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Striking alterations in the soil bacterial community structure and functioning of the biological N cycle induced by *Pennisetum setaceum* invasion in a semiarid environment

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ABSTRACT

The objective of this study was to determine whether native and invasive plants harbor different bacterial communities in their rhizospheres and whether there are bacterial indicator species associated with the invasive rhizosphere. Additionally, physico-chemical, biochemical, and biological properties have been determined in the native and invasive rhizospheres in order to ascertain the relationships between these soil properties and the rhizosphere bacterial communities. We carried out a study in five independent locations under Mediterranean semiarid conditions, where the native *Hyparrhenia hirta* is being displaced by *Pennisetum setaceum*. Partial 16S rRNA genes of the rhizosphere bacterial communities were amplified and 454-pyrosequenced. Principal coordinate analysis revealed differences in the composition and structure of the rhizosphere bacterial communities between native and invasive plants, the values of the richness index being higher in the invasive microbial community. Rhizosphere microbial community structure was also influenced by invaded location. The indicator species analysis showed a higher number of indicators for the invasive community at all the taxonomic levels studied, the genus *Ohtaekwangia* being the most abundant indicator. As shown by canonical correspondence analysis, the protease and dehydrogenase activities and soil respiration were related to the rhizosphere bacterial community of invasive plant. However, only protease activity was significantly affected by the plant type, being higher in the invasive plant rhizosphere. Our results show that *P. setaceum* invasion has produced an intense interaction with the soil bacterial community, shifting its structure, composition, and protease activity related to N cycling, which may be altering the function of the invaded ecosystem.

1. Introduction

Invasive alien species are non-native organisms which are introduced by human action outside their natural range and are able to establish and spread, causing ecological and economic damage (Sundseth, 2014). They have been included among the five main causes of biodiversity loss by the Secretariat of the Convention on Biological Diversity (2014), along with direct habitat destruction and fragmentation; overexploitation of biological resources; pollution; and climate change and acidification of the oceans. The ecosystem is altered not only by direct competition of exotic with indigenous species but also by modification of the structure and functioning through different processes such as productivity, decomposition, hydrology, geomorphology, fire regimes, and nutrient cycling (Vitousek et al., 1997; Kourtev et al., 2002; Levine et al., 2003; Vila and Ibanez, 2011). This leads to biodiversity losses, which have a negative repercussion on the ecosystem services, economy, human health and well-being (Diaz et al., 2006).

Plant invasions have been a target of research for decades and have been addressed by different approaches, including evolutionary, biogeographical, and ecophysiological analysis, among others (Gordon, 1998). In order to identify plant characteristics which serve as predictors for invasiveness and to find and optimize

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solutions, various hypotheses have been postulated to explain the success of invasion (Hierro et al., 2005), and several functional traits of successful alien plants have been described (van Kleunen et al., 2015). On the other hand, it has been demonstrated that invasive plants can exert profound impacts on invaded ecosystems altering soil physical-chemical (Kourtev et al., 1998), chemical (Dassonville et al., 2008; Chacón et al., 2009), physical (Eviner and Chapin, 2002) and biological properties (Souza-Alonso et al., 2014). Moreover, several studies have been addressed to know how the rhizosphere microbiota is related to plant invasiveness since that the plant-microbe interactions can be decisive for the successful establishment of a plant community in a given ecosystem (Kourtev et al., 2003; Batten et al., 2007; Souza-Alonso et al., 2015; Medina-Villar et al., 2016). The interactions established between the invasive plants and soil native biota can lead to positive feedback processes that reinforce the invasion and limit the resistance and resilience to the invasion of the affected ecosystems. In relation to this, van der Putten et al. (2007) reviewed the ecological interactions between invasive plants and pathogens, symbionts, or saprophytic soil microbes, pointing out the importance of these relationships in environmental function and sustainability.

It has been documented that one of the potential strategies that invasive plants employ to ensure success is the alteration of C and N cycles in different ways, such as modification of the soil microbial community structure (Ehrenfeld, 2003; Liao et al., 2008; Dassonville et al., 2011) or increase in the abundance of bacterial populations responsible for N cycling (Rodrigues et al., 2015; McLeod et al., 2016). The effect of plant invasion on soil microbial community functions has been previously evaluated measuring microbial enzymatic activities responsible for soil organic matter decomposition and nutrient cycling (Allison et al., 2006; Souza-Alonso et al., 2014). The assessment of the microbial function of invaded ecosystems can contribute to a greater understanding of the mechanisms of invasion as well as to a better estimation of its impact on environmental function.

Previously, the effects of exotic invasive plants on the soil microbial community structure have been assessed by measuring phospholipid fatty acids (Kourtev et al., 2003; Batten et al., 2007). In the last few years, the development of high throughput sequencing methods for metagenomics has provided the tools suitable for the study of soil microbial communities (Coats and Rumpho, 2014). Some of these molecular methodologies have implemented to identify how exotic invasive plants modify soil microbiota and how these microorganisms enhance the invader's superiority (Coats et al., 2014; Slabbert et al., 2014). However, to the best of our knowledge, the use of these approaches to evaluate invaded Mediterranean semiarid ecosystems has only been reported in a study simulating the process of exotic plant invasion in an experimental semiarid grassland (Carey et al., 2015). The climate in these areas is characterized by low rainfall rates with frequent drought periods, which lead to severe erosive processes and nutrient scarcity. Such restrictive circumstances limit the formation of a vegetation cover (Caravaca et al., 2003) and result in a fragile ecosystem which becomes especially exposed to the inherent risks of global change. However, this harsh environment is suitable for the naturalization of *Pennisetum setaceum* (Forssk.) Chiov, a C4 perennial species of the Poaceae native to Northeast Africa and with rapid growth and abundant seed production (Milton, 2004; Rahlao et al., 2014). Owing to its ornamental potential, it has been introduced and become invasive in USA, Australia, South Africa, and Italy (Milton, 2004; European and Mediterranean Plant Protection Organization (EPPO), 2009). In Spain, it is especially aggressive in the Canary Islands, where it is present in protected areas and is replacing endemic species (Gonzalez-Rodriguez et al., 2010), but it can also be found in the South of the Iberian Peninsula (Sánchez Gómez et al., 2003; Devesa Alcaraz and Arnelas, 2006) where it has escaped into the wild and is threatening the native flora. *Pennisetum setaceum* exhibits a certain preference for anthropogenic and disturbed environments like roadsides. No previous studies on the *P. setaceum* rhizosphere microbiota have been performed to date in spite of the relevance of this information to a better understanding of the ecology of this plant and its colonization mechanisms. Likewise, *P. setaceum* rhizosphere metagenomics could be employed to identify the most susceptible habitats and to design effective control strategies which take into account all the factors involved in its invasive behavior.

We proceeded from the hypothesis that the invasive plant may selectively alter soil biota, independently of the characteristics of invaded site, harboring in its rhizosphere a microbial community endowed with an advantageous functional capacity with regard to nutrients cycling in comparison to that of native rhizosphere. Therefore, this study aimed to assess the differences between the rhizosphere bacterial communities of the exotic *P. setaceum* and the native *H. hirta*, formerly predominant but now displaced by the invader, in five independent locations exposed to Mediterranean semiarid conditions. Additionally, measurements of soil chemical and physico-chemical properties and soil enzymatic activities have been included in this research, to analyze the possible changes driven by the invasive plant and to relate them to the composition and structure of the rhizosphere bacterial communities.

2. Materials and methods

2.1. Plant species, study sites and sampling

Pennisetum setaceum is a perennial bunchgrass with C4 metabolism that can perpetuate itself through apomictic reproduction. Its seeds, which are produced in abundance, are wind dispersed (Rahlao et al., 2014). It is drought tolerant and exhibits a wide phenotypic and reproductive plasticity (Williams et al., 1995). In Southeast Spain, it is usually used as an ornamental plant in public gardens and roundabouts, so it can easily naturalize in the surrounding ruderal habitats - where it competes with the native population of plants. One of these co-occurring natives is *Hyparrhenia hirta*, a C4 perennial bunchgrass very resistant to drought periods and native to the Mediterranean region (Clayton, 1969). It forms dense stands in disturbed areas such as uncultivated lands and roadsides, protecting the soil and stabilizing eroded places. The reproduction strategies for this plant are very similar to that of *P. setaceum*.

We selected five different locations highly invaded by the invasive *Pennisetum setaceum* (about 70%) for less than 10 years, where the dominant native species was *Hyparrhenia hirta* and now forming mixed stands with both plant species (Table 1). All the locations were subjected to a Mediterranean semiarid climate (mean annual temperature around 18 °C and average annual rainfall around 265 mm) but with different edaphic characteristics. The soils from the locations “La Alcayna” (A), “El Tiro” (T) and “Espinardo” (E) are shallow anthropogenic soils taken from roadsides. These soils are difficult to classify because they were highly disturbed during road construction as well as they occasionally receive sediments dragged by the rains. Soil from the watercourse “Rambla de Corvera” (C) is a Calcaric Regosol (IUSS Working Group WRB, 2015) developed on unconsolidated materials with a weakly developed mineral A horizon, whereas the soil from Santa Pola (S) taken in a coastal plain is a Gleyic Solonchak (IUSS Working Group WRB, 2015) predominantly sandy. In these habitats, the vegetation is mainly formed by ruderal herbaceous plants. At each location, three (2 m by 2 m) sampling plots where both species grow and separated by 10 m were established. Within each plot, we collected