



## Applying predictive models to decipher rhizobacterial modifications in common reed die-back affected populations



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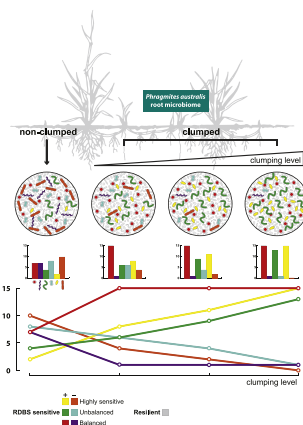
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### HIGHLIGHTS

- Die-back of *Phragmites australis* is a still obscure syndrome.
- Root microbiota composition was shown to be affected by both sites and health status.
- A plant health status x site x microbiota interaction was defined.
- Root microbiota composition was shown to be predictable from plant health status.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The microbiota inhabiting the soil, as well as the rhizosphere, represents a key determinant of several plant functions. Like for humans, dysbiosis of the plant-associated microbiota may be a co-causal agent in disease with still obscure etiology. In the last decades, the common reed *Phragmites australis* has been deeply studied for its disappearance from natural stands, but no clear causative agents have been identified and no laboratory models of such “reed die-back syndrome” (RDBS) have been developed. In this study, we try to shed light on the RDBS, by comparing the rhizosphere microbiota of five Italian *P. australis* populations with different degrees of decline. Results obtained showed a biogeographical meaningful pattern of rhizosphere microbiota, coupled with an impact of RDBS. Obtained data allowed to construct a two-steps predictive model which enabled the prediction of the plant health status from the microbiota taxonomic composition, independently from their geographic location. In conclusion, this study represents one of the first overviews that statistically links RDBS to alteration of rhizosphere microbiota and suggests a model for the analysis of plant-bacteria relationships in nature.

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## 1. Introduction

*Phragmites australis* (Cav.) Steud. is a cosmopolitan species that colonize several habitats such as river banks, lake shores, marshlands, bogs, freshwater or brackish waters, and forming dense and poor-in species stands (Landucci et al., 2013; Lansdown, 2015; Packer et al., 2017). Reed beds play an important role in the water quality control, in the shore stability improvement and in the protection of fauna (Kiviat, 2013; Ostendorp, 1993). Moreover *P. australis* is widely used in waste detoxification, contaminant sequestration and phytoremediation and it provides many cultural (e.g. ecotourism, education) and provisioning (e.g. pharmaceuticals, fibre, energy) services (see (Kiviat, 2013) for details).

Since the second half of the last century, frequent cases of common reed die-back (hereafter RDBS), defined by van der Putten in 1997 (Van Der Putten, 1997) as “a visible abnormal and non-reversible spontaneous retreat, disintegration or disappearance of a mature stand of common reed (*Phragmites australis*) within a period not longer than a decade”, have been reported. At the beginning, the common reed decline was recorded in several European wetlands (Ostendorp, 1989) and appeared limited to Central and Northern Europe (Van der Putten, 1994). Recently, it has also been reported in several wetlands of the Mediterranean basin (Fogli et al., 2002; Gigante et al., 2011, 2014; Lastrucci et al., 2016, 2017).

It is well known that RDBS engenders consistent modifications in terms of physiological and histo-anatomical characters (Lastrucci et al., 2017; Reale et al., 2012). In particular, some macro-morphological and demographic traits, such as reduced culm diameters and increased culm density, seem to be strictly related to the RDBS (Gigante et al., 2014; Lastrucci et al., 2016; Reale et al., 2012). Based on previous studies, a typical (and more evident) trait that unequivocally marks the phenomenon of common reed decline, consists in the occurrence of the clumping habit (Armstrong et al., 1996b; Clevering, 1997), caused by the loss of apical dominance and the development of dormant lateral buds, leading to the formation of peculiar tufts of stems. This trait has been found related to the suffering in affected reed stands in all the previous studies on RDBS from central Italy (Gigante et al., 2011, 2014; Lastrucci et al., 2016, 2017).

Many factors have been suggested among the possible causes of this reed die-back syndrome, like changes in the water table and subsequent alteration of the flooding period, bank erosion, water and sediment chemism, excessive accumulation of litter, reducing soil conditions, phytotoxicity and auto-toxicity, pathogen attacks, grazing (Armstrong et al., 1996a, 1996b; Clevering, 1997; Gigante et al., 2011, 2014; Lastrucci et al., 2016; Nechwatal et al., 2008; Ostendorp et al., 1995; Rea, 1996; Van Der Putten, 1997; Weisner, 1996). The role of the rhizosphere in the die-back occurrence has been long since supposed (Armstrong et al., 1996b; Sorrell et al., 1997) but no specific studies on the possible relationships between rhizobacterial communities associated with vigorous *P. australis* and affected by RDBS have been carried out. In the last decade, several studies focused on the knowledge on microbial communities of *P. australis* (Borruso et al., 2014; Chaturvedi et al., 2006; Ma et al., 2014). Only recently, an indication that oomycetes may play a role in RDBS has been published (Cerri et al., 2017), however, no indication has been reported on a possible role of prokaryotic microorganisms.

The rhizosphere, defined as “the narrow zone surrounding and influenced by plant roots”, was considered a hot-spot for diversity of microorganisms and was ascribable to one of the most complex ecosystems of the earth (Raaijmakers et al., 2009). An increasing amount of literature (De Deyn et al., 2004; Hooper et al., 2005; Lau and Lennon, 2011; Van Der Heijden et al., 2008) has demonstrated direct and/or indirect effects of plant-microorganism association on some plants “functional traits” such as seed germination, seedling vitality, plant nutrition, development and productivity. However, for the vast majority of plant-associated microorganisms, there is a limited knowledge of their impact

on plant growth, health, and disease. Hence, deciphering the plant microbiome is critical to identify microorganisms that can be exploited for improving plant growth and health. The rhizosphere of reed has been extensively explored in relation to the adaptation of the plant to toxic and harsh conditions and to decontamination and specific bacterial activities have been detected (for examples see (Chaturvedi et al., 2006; Toyama et al., 2011; Van Der Nat and Middelburg, 1998)). Indeed, it is well known that rhizosphere microbiota is strongly shaped by root exudates, and that a strong interplay between the root and its microbiota is present. Concerning the rhizosphere bacterial communities, assemblage of taxa related to sulphur and nitrogen cycles have been detected, in relation to the submerged (i.e. hypoxic) conditions of the rhizome (Ravit et al., 2007; Ruiz-Rueda et al., 2009). More recently, to the dependency between reed genetic lineage and rhizosphere microbiota has been also highlighted (Bowen et al., 2017). Moreover, the adaptation of the reed to salinity, possibly related to rhizosphere bacterial communities has been investigated with 16S rRNA-based metabarcoding of the rhizosphere microbiota (Borruso et al., 2014; Canfora et al., 2014; Nie et al., 2009; Wang et al., 2007). Finally, preliminary reports of variability of endophytic and rhizospheric bacterial communities due to plant variety have been published (Li et al., 2010; Zhang et al., 2013). A work performed on healthy and declining reed stands in lake Velencey in Hungary (Micsinai et al., 2003), failed to find evidences of the presence of plant-pathogenic bacteria in the rhizosphere microbiota, suggesting that no single or known plant-pathogenic bacteria are associated with declining reed stands. It is then conceivable that, as for other complex diseases (e.g. human dysbioses, see for instance (Bacci et al., 2016; Deng et al., 2017)) a change in the overall microbiota may be linked to reed decline. To the best of our knowledge, no experiments under controlled conditions were performed on the relations between RDBS and rhizospheric bacterial community primarily (i) because of limitations in the identifications of the factors promoting the syndrome and secondarily (ii) for the extremely long time needed for the eventual execution of the laboratory phase. For these reasons, only an in-situ survey can lead a more detailed description of reed die-back syndrome. Here, combining predictive modelling with the taxonomic characterization of the microbiota through 16S rRNA gene amplicon sequencing, we aimed to shed light on the possible contribution of the rhizosphere microbiota to the RDBS. We designed an experimental scheme comprising five geographically well differentiated lakes, each including in turn 16 areas characterized by different health status of reed-dominated stands. The aims of the work were: 1) to decipher the microbial components of rhizosphere in each common reed stand 2) to investigate the presence of relationships between RDBS and the rhizosphere microbiota in terms of shifts from bacterial core community to bacterial related with RDBS.

## 2. Materials and methods

### 2.1. Sampling design

The sampling has been performed on 40 plots (1 m × 1 m in size) randomly selected within five wetlands of central Italy (Fig. 1).

The number of clumped culms in each plot, here referred to as the tufted growth form of common reed, has been used as an indicator of RDBS incidence. This above-ground feature has been accurately described in literature and strongly related to the reed die-back (Armstrong et al., 1996b; Clevering, 1997). It was defined as a “late feature associated with die-back”<sup>15</sup> and thus it was used in this work as an indicator of plant disease progression. Clumping levels were calculated in each plot as the ratio between the number of culms in each clump (when present) and the total number of culms in the plot:

$$C = \left( \frac{N_{cb}}{N_c} \right) \cdot 100$$

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