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Distance from the sea as a driving force of microbial communities under water potential stresses in litters of two typical Mediterranean plant species

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ABSTRACT

Though many studies have focused on the incidence of drought, little attention has been paid to osmotic stress and to how the interaction of the two stresses impacts microbial functioning. Moreover, deciphering how certain environmental factors such as distance from the sea or type of litter may shape microbial responses to these stresses is of huge importance. The objective of this study is to shed light on the impact of matric and osmotic stresses (combined or not) on litter microbial communities potentially shaped differently by either plant species or distance from the sea. Two Mediterranean plant species, *Cistus albidus* L. and *Pistacia lentiscus* L., collected from both inland and coastal areas, were used to set up a total of 72 mesocosms (4 mesocosms × 3 types of litters (pure or mixed) × 2 sites (coastal and inland) × 3 types of stress). A first set of twenty four mesocosms 'control' (4 mesocosms × 3 types of litters (pure or mixed) × 2 sites (coastal and inland)) were maintained under favourable conditions for 60 days (25 °C, 600 $g\cdot kg^{-1}$ water content). For matric stress, twenty four mesocosms were subjected to 5 drying/rewetting cycles (7 days at 25 °C/7 days at 600 $g\cdot kg^{-1}$ water content and 25 °C). For osmotic stress, twenty four mesocosms received 10 mg of chlorine ions per g of litter using NaCl and then were incubated at 600 $g\cdot kg^{-1}$ water content and 25 °C for 60 days. The last twenty four mesocosms were subjected to the combined drought and salt stresses for 60 days. Resistance to added osmotic stress was also tested. Catabolic diversity assessed via Biolog Ecoplates, was higher in coastal than in inland areas after drought and combined stresses. Moreover, catabolic profiles were shaped differently after stresses depending on the litter type. Basal respiration withstood an added severe drought stress better in microbial communities previously subjected to combined stresses. Resistance to an added osmotic stress was higher in inland litters previously subjected to salt stress. Microbial communities from the coastal area were more able to maintain their catabolic potential and thus to withstand these stresses. Combined stresses and osmotic stress reinforced resistance to added severe drought or osmotic stress respectively. Thus our findings reveal that microbial communities previously subjected to water potential stresses were more capable to overcome additional stresses of similar nature, suggesting adaptation mechanisms to such type of stresses.

1. Introduction

Organic matter turnover is a crucial ecological process mainly realized by microbial communities via the exocellular enzymes they produce. Litter functioning is driven by various biotic and abiotic factors such as pedoclimatic conditions and nutrient quantity and quality, which are known to strongly determine the composition and structure of microbial communities (Coûteaux et al., 1995; Hättenschwiler et al., 2005). In the Mediterranean region, environmental conditions are particularly extreme. Soils are poor in organic matter, litters contain large quantities of recalcitrant phenolic compounds and the climate is characterized by severe summer drought (Pons and Quézel, 1998). Moreover, in coastal environments, such stresses are reinforced by the wind regime, which exacerbates desiccation, as well as more intense sunlight. Additional stresses, such as osmotic stress due to sea spray exposure, can also impact litter functioning (Qasemian et al., 2014). However, while many studies have examined litter functioning in inland areas under a Mediterranean climate (Fioretto et al., 2007, 2009; Papa et al., 2008), few have investigated whether microbial communities in coastal environments may be even more weakened by these additional and intensified pressures. Moreover, little is known about how the high salt concentrations in these environments may shape microbial communities and their tolerance to osmotic stresses (Rath and Rousk, 2015).

Both matric and osmotic stresses strongly influence microbial communities and their activities (Berard et al., 2011; Sardans and

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Penuelas, 2005; Sardinha et al., 2003) by drastically reducing water availability. This limits microbial growth, enzyme production and can even modify the protein conformation of enzymes (Wehtje *et al.*, 1997). The impact of drying and rewetting events on litter microbial activities has been investigated extensively. The general conclusion is of a priming effect linked to the mineralization of labile carbon from cell lysis, aggregate breakdown or changes in microbial community structure (Borken and Matzner, 2009; Kakumanu et al., 2013). When the frequency of such events increases, these flushes of microbial activities decrease because of rarefaction of labile carbon amount and a specialization of microbial communities less sensitive to matric stress, with a constant microbial biomass and a low-level respiration rate (Butterly et al., 2009; Fierer et al., 2003). Microbial adaptation to osmotic stress is also observed with accumulation of osmolytes inside the cell (Kakumanu and Williams, 2014), which prevents cell lysis, but which is an energy-consuming process (Schimel et al., 2007). The recent studies of Chowdhury and Marschner (2011), Chowdhury et al. (2011a,b) have taken the first steps towards providing information about soil responses to both osmotic and matric stresses, suggesting a threshold of tolerance to decreasing water potential in the physiological mechanisms underlying microbial adaptation. However, the combination of such stresses typical of coastal environments and their effect on microbial functioning, have not yet been thoroughly evaluated.

We hypothesise here i) that litter microbial communities selected by coastal area conditions may be better resistant to water potential stresses than those from inland areas and ii) that different types of litter (i.e. different organic matter quality and quantity) may also influence the diversity of microbial communities and thus their tolerance of these stresses (Yuste et al., 2011). For instance, availability of nutrients, and recalcitrance of organic matter are supposed to strongly modify microbial diversity or the balance between bacteria and fungi, which are known to exhibit various tolerances to matric stresses. To test these hypotheses, we selected two Mediterranean plant species, *Cistus albidus* L. and *Pistacia lentiscus* L., collected both from inland and from coastal areas. Mesocosms were set up for each litter and for a mixture (50/50) of the plant species under study. These mesocosms were then subjected to drought, salt or combined stresses, mimicking conditions recorded in the coastal sampling area (Qasemian et al., 2014). Resistance to added osmotic stress or severe drying and rewetting events was also tested. Our objectives were i) to compare the responses of litter microbial communities to salt, drought or combined stresses found in coastal areas, ii) to determine the influence of type of litter (*C. albidus* L. vs *P. lentiscus* L.) and of distance from the sea (inland vs coastal litters) on microbial responses to stresses, iii) to test whether pre-exposure to stresses selected microbial communities more adapted to added osmotic stress and severe drying and rewetting events.

2. Materials and methods

2.1. Site description and litter sampling

Six sampling sites were chosen in the peri-urban area of Marseille on the French Mediterranean coast (43°12′38.80″N; 5°21′19.53″E). They included three coastal sites and three inland sites located respectively 30 m and 6 km from the sea (Qasemian et al., 2014). The distance between sites, whether coastal or inland, is 2 km at least, far exceeding the spatial dependence of most microbiological properties in soil (Nannipieri et al., 2003). The climate is Mediterranean, with a mean annual precipitation of 520 mm and mean monthly temperatures ranging from 7.2 °C in January to 24.8 °C in June. These sites (25–230 m in elevation) present similar exposure (South), slope (10–15%) and soil type (Calcaric Leptosol according to IUSS Working Group WRB, 2006). Shrub canopy cover is about 80% and characterized by *Quercus coccifera*, *C. albidus*, *Cistus salvifolius*, *Rosmarinus officinalis*, *P. lentiscus*, *Rhamnus alaternus*, *Asparagus acutifolius* and *Phillyrea angustifolia*.

At each site, composite sampling was performed in November 2011 from litter (horizon Ol) of *P. lentiscus* and *C. albidus*. *P. lentiscus* is a thermophilic evergreen shrub, with sclerophyllous leaves, native throughout the Mediterranean region since it is highly tolerant to poor calcareous soils and to severe drought and is particularly widespread in seaside stony areas (García-Fayos and Verdú, 1998). *C. albidus* is a pioneer semi-deciduous shrub with haired leaves, also widely found in xeric Mediterranean soils (Oliveira and Peñuelas, 2000). *C. albidus* and *P. lentiscus* are the two dominant shrub species both in the coastal and inland areas under study. For each species, twenty samples of litter were randomly collected over a 1000 m^2 area to obtain a composite sample. Contaminating debris (e.g. leaves of other species, including branches and seeds) were removed carefully from each collection. The samples were homogenized and stored in polyethylene bags at 4 °C until mesocosm preparation.

2.2. Mesocosm preparation and experimental set-up

Litter was placed in horticulture pots (10 × 10 × 10 cm) containing either 30 g (dry weight, DW) of *Cistus* litter, *Pistacia* litter or of a mixed *Cistus*–*Pistacia* litter (50/50, DW/DW) per mesocosm. A total of 72 mesocosms were prepared (4 mesocosms × 3 types of litter × 2 sites (coastal and inland) × 3 types of stress). Litter was rehydrated to 600 $g \cdot kg^{-1}$ water content with deionized and sterilized water and the mesocosms were covered with pierced tin foil. The 72 mesocosms were pre-incubated for 30 days under temperature (25 °C) and water content (600 $g \cdot kg^{-1}$) conditions favourable to microbial growth. 600 $g \cdot kg^{-1}$ water content was chosen since this was the maximum water content recorded in litter in the field over a one year period in the coastal areas where samplings were performed (Qasemian et al., 2014). After this period of pre-incubation, a first set of twenty four mesocosms ‘control’ (4 mesocosms × 3 types of litters (pure or mixed) × 2 sites (coastal and inland)) were maintained under favourable conditions for 60 days (25 °C, 600 $g \cdot kg^{-1}$ water content). A second set of twenty four mesocosms (drought stress) were subjected to 5 drying/rewetting cycles. Each drying/rewetting cycle was composed of 2 steps: i) a drying period of 7 days at 25 °C to reach a final water content close to 150 $g \cdot kg^{-1}$ ii) a quick rewetting to recover 600 $g \cdot kg^{-1}$ water content followed by an incubation period for 7 days at 600 $g \cdot kg^{-1}$ water content and 25 °C. A third set of twenty four mesocosms (osmotic stress) received 10 mg of chlorine ions per g of litter using NaCl (EC of 1400 $\mu S \cdot cm^{-1}$), which is the higher concentration found over a one-year monitoring in the coastal areas where samplings were performed as described by Qasemian et al. (2014). Then these mesocosms were incubated under the favourable conditions described above for 60 days. The last twenty four mesocosms were subjected to the combined drought and salt stresses for 60 days. A preliminary experiment showed that the same level of water potential stress assessed via water activity, a_w, (measured with Hygropalm probe, Rotronic, Fisher Scientific, Illkirch, France) was achieved: for matric stress, when 150 $g \cdot kg^{-1}$ water content was reached and for osmotic stress, after NaCl addition to litter at 600 $g \cdot kg^{-1}$ water content, a similar a_w value of 0.8 was obtained. For combined stresses, a_w was measured after NaCl addition to litter at 150 $g \cdot kg^{-1}$ water content and a value of 0.7 was obtained. Thermodynamic water activity is defined as the ratio of water vapour pressure in the system over pure water that at constant pressure and temperature.

After incubation time, a fraction of each sample was dried and ground prior to chemical analysis and all the experiments involving microbial markers started immediately and were performed over one week.

2.3. Plant litter chemical traits

The chemical composition of *Cistus* and *Pistacia* litters collected in coastal and inland sites and used in the mesocosm-scale experiment