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Sporulation and physiological profiles of bacterial communities of three Mediterranean soils affected by drying-rewetting or freezing-thawing cycles

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ABSTRACT

In the global change context, the basal respiration (BR), the estimated number of bacterial spores (SP) and the community level physiological profiles (CLPPs) were investigated in three different Mediterranean soils following different hydric and thermic stress scenarios. The treatments consisted in an increasing number (1, 2, 4, and 7) of drying-rewetting (DRWc) or freezing-thawing cycles (FTc) at 20, 40 or −20 °C. The results highlighted that the different soils responded differently to the same treatment and that the three variables considered were weakly related one to each another. In almost all soils and modalities, the BR increased significantly during the first cycles before decreasing during the last. With regards to SP, it appeared that, for a given soil, the capacity of microbial communities to sporulate and/or germinate can be considerably more influenced by the temperature rather than by the hydric stress. Finally, the CLPPs literally collapsed with the treatment at 40 °C, irrespective of the soil considered. This suggested a progressive replacement of the catabolically diversified original bacterial communities by another showing lower functional diversities.

1. Introduction

Soil microbial communities are known to be sensitive to climate (Chemidlin Prevost-Boure et al., 2011), and it is globally admitted that under Mediterranean climate, the global change will lead to heat and drought waves that will durably affect the terrestrial ecosystems (IPCC, 2014), in part through increased vulnerability of microbial edaphic functions. If respiratory and enzymatic activities of soils subjected to climatic stresses are beginning to be well documented (*e.g.* Guénon and Gros, 2013; Daou et al., 2016; Gao et al., 2016), those regarding the effect of global change on the catabolic functional diversity of soil microorganisms are much rarer. Even more, data about climatic stress simulations on the sporulation abilities of soil microbial communities are still inexistent, despite the importance of sporulating microorganisms in soil functioning. Indeed, among the affected microorganisms, the spore-forming bacteria are known to be particularly abundant in soils and it was demonstrated that their abundance and their specific richness could be influenced by hydric and thermic characteristics of their environment (Périssol et al., 1993; Brunel et al., 1994). Thus, the objectives of this study were to determine how different thermal (- 20 °C, + 20 °C or + 40 °C) and/or hydric stress scenarios, i.e. drying-rewetting (DRWc) and freezing-thawing (FTc) cycles, would influence, negatively or positively, the basal respiration (BR), the estimated number of bacterial spores (SP) and the community level physiological profiles (CLPPs, Biolog EcoplatesTM) of soil microorganisms. We aimed also to determine which climatic factor (DRWc/FTc or temperature) most influences the different microbial variables and if the responses observed are modulated by the origin and the pedological and pedoclimatic characteristics of the different Mediterranean soils investigated.

2. Materials and methods

Mesocosm experiments were conducted (Fig. 1) with three different Mediterranean surface soils (0–5 cm depth, five samples each) collected during February 2014 in the Bouches-du-Rhône,

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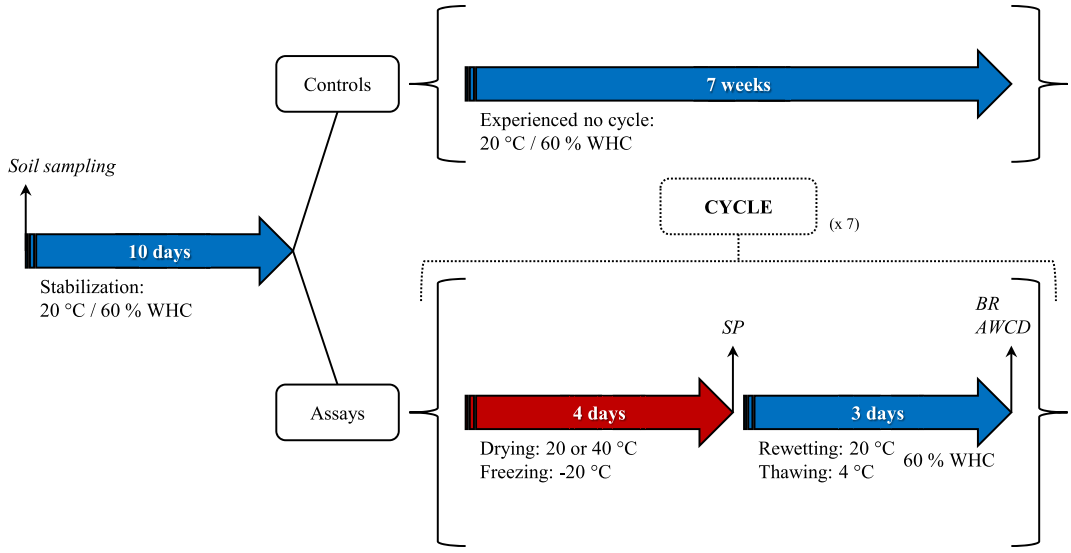

Fig. 1. Design of mesocosm experiments. Samples were incubated for stabilization at 20 °C and 60% WHC for 10 days prior to the experiments. Controls consisted in 5 replicates for each soil (3 soils x 5 samples; 15 controls at total) maintained at 20 °C and 60% WHC for 7 weeks. The different samples subjected to stress scenarios were obtained as follows: 3 soils × 4 cycle treatments (cycle1, cycle2, cycle 4, cycle7) x 3 temperatures (−20 °C, 20 °C, 40 °C) x 5 replicates (180 samples at total). *SP*, the estimated number of bacterial spores was measured after the drying/freezing phase and *BR*, the basal respiration and *AWCD*, the average well color development (Biolog EcoPlates™) were measured after the rewetting/thawing phase. *WHC*, water holding capacity. 195 mesocosms at total.

France. A south oriented soil (S) (43°19′5″ N, 5°40′2″ E, 900 m altitude), a north oriented soil (N), opposite to the south oriented plot (43°19′29″ N, 5°40′23″ E, 700 m altitude), and a riparian forest soil (R) (43°31′11″ N, 5°34′10″ E, 420 m altitude). For each soil, samples were sieved at 2 mm, pooled into one composite sample, and the dry mass (DM) and the water holding capacity (WHC) were determined prior to the experiments. The textures of soils were classified as clay loam (S and N soils, Colluvic Rendzic Leptosols; IUSS, 2006) and silty clay loam (R soil, Calcaric Stagnic Fluvisol; IUSS, 2006). The three soils showed an alkaline pH (7.6), and similar cation exchange capacity (22.8–24.8 $cmol^{+}\ kg^{-1}$ DM) and total nitrogen content (4.4–5.0 $g\ kg^{-1}$ DM). The total organic carbon contents were 55.4, 64.7 and 82.4 $g\ kg^{-1}$ DM, respectively for S, R and N soils. Considering the climatic characteristics of the different plots, the S soil (transition between meso- and supra-Mediterranean bioclimatic stages) was, along the year, subjected to more drastic temperature and relative humidity variations than the R soil (medio-european influences). The pedoclimatic conditions of the N soil (supra-Mediterranean-subhumid bioclimatic stage) appeared as intermediary to the two other soils. See Appendix A for more details about floristic inventories of plots and physicochemical and pedoclimatic characteristics of soils (Daou et al., 2016). The BR was measured according to the technique of Anderson and Domsch (1978), using gas chromatography (Chrompack CHROM 3 – CP 9001, equipped with a Porapak™ column type, under a helium flux of 650 mL h^{-1}). The Biolog EcoPlates™ technique was used according to the protocol of Floch et al. (2011) to determine the metabolic potentialities (*i.e.* CLPPs) of aerobic heterochemoorganotroph communities of soils. After 72 h of growth in microplate, the average well color development (AWCD, *i.e.* average metabolic activity) was calculated according to the method of Garland and Mills (1991) and was used as indicator of the CLPPs (Preston-Mafham et al., 2002). Relative use of several classes of substrates (polymers, carbohydrates, carboxylic acids, amino acids, amines and miscellaneous) was also calculated according to Selmants et al. (2005). To achieve this, each class of substrate was calculated as corrected absorption values, i.e. divided by total absorption in the plate. The number of bacterial spores was estimated by using the most probable number method (MPN) and the algorithm developed by Briones and Reichardt (1999). Briefly, 1 g of soil was suspended in 9 mL of 0.1% sterile sodium pyrophosphate solution, then agitated for 20 min at 50 rpm (*i.e.* 5/6 Hz) and submitted to decimal dilutions with a 0.85% sterile NaCl solution. The different dilutions were thereafter pasteurized at 80 °C for 10 min to eliminate non sporulated microorganisms and to preserve the bacterial spores (Travers et al., 1987). 20 µL of these dilutions were inoculated in 96 wells microplates (Nunc™) filled with 180 µL of nutrient broth medium (NB) at 20 $g\ L^{-1}$ (Biokar Diagnostics). For each dilution, eight repetitions were done and microplates were incubated at 30 °C during 3 days before reading and calculation of the MPN.

Concerning statistics, prior to analyses, the hypotheses of data normality (Shapiro-Wilk test) and variance homogeneity (Bartlett test) were checked. For each soil and temperature, repeated measures analyses of variance (rANOVA) were performed on each microbial variable to test the effect of cycle factor (within-subject factor; four levels: 1, 2, 4, and 7 cycles), at a significance level of $p < 0.05$ ($n = 5$). Thereafter, a *post hoc* test of pairwise multiple comparisons of Tukey (honestly significant difference, HSD) was used to verify whether the various levels of the tested factor were significantly different from each other, at a significance level of $p < 0.05$. For each soil, analyses of variance (ANOVA) were performed on each microbial variable of the last cycles to test the effect of temperature factor (between-subject factor; three levels: −20, 20, and 40 °C), at a significance level of $p < 0.05$ ($n = 5$). Thereafter, a *post hoc* test of comparisons with a control of Dunnett was used to verify whether the various levels of the tested factor were significantly lower (left-tailed test) or greater (right-tailed test) than the control, at a significance level of $p < 0.05$. All these analyses were conducted using XLSTAT software version 2009.3.02 (Addinsoft™, France).

3. Results and discussion

For all soils, results showed that the temperature, the cycles and their interaction had significant effects on BR (Table 1). Moreover,