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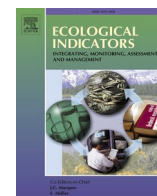
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Litter microbial responses to climate change: How do inland or coastal context and litter type matter across the Mediterranean?

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ABSTRACT

Warmer and drier climates are expected in the Mediterranean basin which is considered as a climate-change hotspot. Here we used climate contrasts across the Mediterranean to mimic climate change effects on litter microbial communities. Litterbags of monospecific (*Pinus halepensis* and *Pistacia lentiscus*) and of binary mixtures of litter (*Pinus/Pistacia*, 50/50) were transferred across contrasted climates between France and Algeria (from a sub-humid to a semi-arid climate). We tested the effect of litter type (species identity, litter-mixing) and of environmental context (coast/inland) on microbial responses to more constraining climate conditions. After 12-months of field incubation, litter chemical properties (C/N and solid-state ¹³C NMR) and microbial markers (active microbial biomass (MB), basal respiration (BR), bacterial catabolic and genetic structures (BIOLOG and T-RFLP) were characterized. After transfer, catabolic and genetic structures were different from those of both French and Algerian control and responses depended on the context and on litter type: stronger modifications were observed in inland context, except for microbial communities of *Pistacia lentiscus* litter (weak variations). However, MB and BR decreased in response to transfer for this litter type. On the other hand, and for either inland or coastal context, MB of *Pinus halepensis* litter did not vary in response to transfer and BR decreased reflecting microbial performance in sustaining biomass and limiting C losses through respiration. Here, litter mixture did not mitigate the effect of climate stress on microbial communities neither in inland nor in coastal context: lower MB and BR were detected after transfer in inland context whereas lower MB was accompanied by a higher BR in coastal context, reflecting an increase in CO₂ release for a weaker amount of microbial biomass. This study shows that microbial responses to climate change vary with litter type and also with the environmental context.

1. Introduction

Soil microorganisms are key players of terrestrial ecosystem functioning. They contribute to the sustainability of nutrient cycling through organic matter decomposition. During this process, carbon is stored or lost from soils and this greatly depends on how microorganisms partition organic matter carbon between respiration and biomass growth (Bardgett, 2005; Manzoni et al., 2012; McGuire and Treseder, 2010). Thus, understanding microbial functioning is crucial for a better prediction of carbon cycle feedbacks on climate (Aerts, 1997; Allison and

Treseder, 2008).

Climate conditions (mainly temperature and moisture) are recognized as major drivers of microbial activities (Curiel Yuste et al., 2007) and community structure (Curiel Yuste et al., 2011). In the context of the ongoing climate changes (warming and disturbance of precipitation dynamics), increased temperatures are supposed to stimulate microbial enzymatic activities and accelerate litter degradation (Erhagen, 2013). However, drier conditions are expected to inhibit microbial activities -by limiting both protein expression and interaction with the substrate (Allison and Treseder, 2008)- and to reduce microbial biomass (Sheik

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et al., 2011). The final impact of climate change on soil functioning is thus not easily predictable. It has been shown that under warming, the water budget of an ecosystem controlled microbial abundance and diversity (Sheik et al., 2011). How changes in temperature or moisture would affect litter decomposition is supposed to depend on the environmental context, since effects may vary according to the climate constraints of a given ecosystem (Prescott, 2010). Water availability is the main environmental constraint in Mediterranean ecosystems due to intrinsic combination of high temperatures and low rainfall in summer (Larcher, 2000). The Mediterranean region is thus particularly vulnerable to the effects of climate change (Schroter, 2005). It is indeed considered as one of the most sensitive regions to global change and is defined as a “climate change hot-spot” (Giorgi, 2006). Warming in the Mediterranean basin exceeds global trends of the whole planet: compared to preindustrial temperatures, annual mean temperature has already risen by 1.4 °C in this region, *versus* an average increase in 1.1 °C worldwide (Cramer et al., 2018). This stresses the necessity to investigate consequences on soil microbial decomposers in this sensitive region where warmer and drier conditions are expected (Cramer et al., 2018; Giorgi and Lionello, 2008).

Coastal environments are obviously emblematic of the Mediterranean and it is noteworthy that soil microbial functioning in these complex and heterogeneous interfaces is still poorly documented (Qasemian et al., 2014). Recent studies revealed differences in microbial activity responses to drought stress according to the environmental context of origin (coast vs inland) and underlined that pre exposure to stresses linked to coastal areas (osmotic stress via sea spray exposure, wind regime and desiccation, higher temperatures) led to more efficient responses to drought stress (Boukhoudou et al., 2016; Farnet Da Silva et al., 2016; Kheir et al., 2019). Thus, these studies highlighted the need for considering coastal environments when investigating microbial structural and functional responses to climate change.

Organic matter quality and quantity (*via* litter inputs) are also recognized as main drivers of microbial structure and functioning (Setiawan et al., 2016; Zimmer, 2002). For instance, litter of good quality (rich in nutrients and labile carbon) are supposed to enhance microbial activities and litter decomposition (Berg, 2014; Setiawan et al., 2016). Moreover, research has shown that litter-mixtures of different plant species generally decompose differently than monospecific litter (Gartner and Cardon, 2004). It has been suggested that plant litter diversity regulated microbial community structure and activities (Hättenschwiler et al., 2005; Santonja et al., 2017b) and that diversity of nutrient resources found in litter mixtures favored microbial biomass and diversity (Chapman and Newman, 2010; Meier and Bowman, 2008). Some observations indicated that litter mixing mitigate drought effect on microbial decomposers (Santonja et al., 2017a). Studies are showing that these effects depend on species identity and litter quality (Brunel et al., 2017; Kheir et al., 2019).

Since soil microbial communities and their activities can be considered as good indicators of soil functioning (Qasemian et al., 2011), understanding how these processes may be hindered by climate change is thus of great importance. Mediterranean ecosystems have been considered as an exceptional playground to test microbial responses to increased aridity (Curiel Yuste et al., 2014). However, to our knowledge, though a wealth of studies used litterbag approach, no study attempted to mimic the effect of climate change by using a latitudinal climate gradient across the Mediterranean basin yet. In this context, our study aimed to test responses of litter microbial communities (microbial active biomass, basal respiration, catabolic and genetic structures) to an increase in aridification in the Mediterranean basin. We used an original *in natura* approach involving litterbag transfer through contrasted climates between Northern and Southern Mediterranean (sub-humid climate in France vs semi-arid climate in Algeria). Moreover, this study aimed to determine whether litter microbial responses after transfer may vary according to i) local conditions using two environmental contexts i.e. coastal and inland areas and/or ii) the type of litter (monospecific vs

admixture; plant species identity). For that purpose, litter of two plant species commonly found in Mediterranean coastal and inland areas and in both countries (France and Algeria) were studied: *Pinus halepensis* (a resinous species), *Pistacia lentiscus* (a leafy species), and their admixture (50/50). These litter samples were packed into litter bags which were left in their ‘home’ sampling area as controls (i.e. French and Algerian inland and coastal areas), while a second set of litter bags were transferred from France to Algeria sites (for both coastal and inland) to test the responses of microbial communities subjected to more drastic environmental conditions. After a 1-year of field-incubation, different scenarios can be expected, mainly: i) a shift in the initial microbial communities (potential selection of more adapted microorganisms) inducing -or not- a change in microbial activities and biomass; ii) a rapid colonization by autochthonous microorganisms leading to similar responses comparing to Algerian controls.

2. Materials and methods

2.1. Site description and litter sampling

Sampling sites were selected throughout a latitudinal gradient across the Mediterranean basin. Both inland and coastal areas in France are located in region Sud Provence-Alpes-Côte d’Azur (South-Eastern France) in the Bouches-du-Rhône and belong to the sub-humid bioclimatic stage (Quezel, 1979; Quézel and Médail, 2003). Sampling sites are located in the Massif de la Trevaress (N43°38’33.97”; E5°25’58.36”) for inland area and in the *peri*-urban area of Marseille on the French Mediterranean coast (N43°12’38.80”; E5°21’19.53”) for coastal area.

Both inland and coastal areas chosen in Algeria are located respectively in the Wilaya of Saida and in the Wilaya of Tlemcen (North-West of Algeria) and belong to the semi-arid bioclimatic stage (Ghezlaoui and Benabadji, 2017; Zouidi et al., 2019). Sampling sites were located in the Massif of Jebel Sid Ahmed Zeggai (N34°46’33.71”; E 0°04’02.47”) for inland area and in the *peri*-urban area of Tlemcen on the Algerian Mediterranean coast (N35°06’27.90”; W1°50’22.78”) for coastal area.

In both countries, five sampling sites (400 m²) were chosen for each context i.e. inland and coastal (a total of 20 sites). The distance between each site is 2 km at least. The sites selected had similar pedoclimatic and topographic features: elevation between 500 and 900 m for inland sites and < 100 m for coastal sites, South exposure (except for Algerian coastal sites at West exposure), slope (10–15%) and soil type (Calcaric Leptosol according to IUSS Working Group W.R.B., 2006). For each site, maximal and minimal temperature and the relative humidity of air were measured *in situ* by EL-USB 2.0 probe (Conrad) over a one-year period. Annual precipitation, mean annual temperature, and precipitation of driest and wettest months were collected from Worldclim (Worldclim, 1970–2010, Hijmans et al., 2005) to highlight the cross-Mediterranean climate contrasts between France and Algeria (Table 1). While several numerical indices have been proposed to measure aridity (Stadler, 1987), annual precipitation remains the simplest aridity index used by the Intergovernmental Panel on Climate Change: an arid region receives <250 mm of annual precipitation whereas a semi-arid region receives between 250 and 500 mm of annual rainfalls (IPCC, 2007; Maliva and Missimer, 2012). Thus, with 350 mm and 394 mm of annual precipitation respectively for inland and coastal area in Algeria vs 643 mm and 602 mm of annual precipitation respectively for inland and coastal area in France, Algerian study sites reflect indeed the more arid conditions. This is also supported by higher mean annual temperatures, more extreme hot season, and lower relative air humidity in the Algerian sites (Table 1).

In March 2017, for each species and for each site, twenty samples of litter were randomly collected over a 1000 m² area to obtain a composite sample. 2 kg of litter of each species were sampled from the very upper layer of horizon O1 to ensure homogenous characteristics of the litter collected and this composite sample was used to prepare litter bags. Contaminating debris (e.g. leaves of other species, including

Table 1

Climate parameters recorded in the field over the 1-year incubation in France and Algeria (from both inland and coastal contexts) and from Worldclim data.

	France		Algeria	
	Coastal	Inland	Coastal	Inland
Area location	N43°12'38.80" E5°21'19.53"	N43°38'33.97" E5°25'58.36"	N35°06'27.90" W1°50'22.78"	N34°46'33.71" E0°04'02.47"
<i>Environmental data monitored in situ (April 2017– March 18)</i>				
Minimal temperature of the coldest month (°C)	0	−6.5	5	−5.5
Maximal temperature of the hottest month (°C)	44.5	38	48.9	49
Annual mean temperature (°C)	18.64	15.15	21.22	17.94
Minimal relative humidity of air (the driest month)	24	25.5	12.1	5.5
<i>Worldclim data</i>				
Annual mean temperature (°C)	14.1	13.1	18.4	15.5
Annual precipitation (mm)	602	643	394	350
Precipitation of the driest month (mm)	13	16	7	8
Precipitation of the wettest month (mm)	85	85	65	45

branches and seeds) were removed carefully from each collection. The samples were stored at 4 °C for one week until litter bag preparation.

2.2. Litterbag preparation and experimental set-up

A total of 90 litterbags were prepared (5 sites × 3 litter types × 2 environmental contexts × 3 treatments). Litter was placed in litterbags (30 × 10 cm, 2 mm rigid nylon mesh) containing 30 g (dry weight, DW) of litter per litterbag. A total of 60 litterbags were then placed in their 'home' area in either coastal or inland area in France and Algeria as controls and 30 litterbags were transferred from France to Algeria (15 in the Algerian coastal area and 15 others in Algerian inland). After 12 months in the field, litterbags were collected. An aliquot of each sample was kept after drying and ground prior to chemical analysis. For microbial analyses, all the experiments started immediately and were performed over one week. For T-RFLP, samples were frozen and analyzed one month later.

2.3. Litter chemical properties

The chemical properties of litter from *P. halepensis*, *P. lentiscus* and their admixture were analyzed. Total organic carbon (TOC) and nitrogen (N) contents were analyzed by combustion in an Elemental Analyzer (Flash EA 1112 series ThermoScientific, Illkirch-Graffenstaden, France). Moreover, litter chemical properties were also analyzed by solid-state ¹³C NMR. Spectra were obtained on a Bruker Avance III HD –400 MHz NMR spectrometer operating at a ¹³C resonance frequency of 100.7 MHz and using a commercial Bruker double-channel probe. A single measurement was performed for each litter bag. About 100 mg of sample were placed in zirconium dioxide rotors of 4-mm outer diameter and spun at the Magic Angle Spinning (MAS) rate of 10 kHz. The Cross Polarization (CP) technique was applied with a ramped 1H-pulse starting at 100% power and decreasing to 50% during contact time (2 ms) to avoid Hartmann-Hahn mismatches. The experiments were performed at ambient temperature and 4 K scans were accumulated using a delay of 2.5 s, giving an experimental time of 3 h. The ¹³C chemical shifts were referenced to tetramethylsilane and calibrated with a glycine carbonyl signal, set at 176.5 ppm. The ¹³C NMR spectra were divided into 7 chemical shift regions according to Knicker and Lüdemann (1995): i.e. alkyl C (0–45 ppm), methoxyl C or N-alkyl C (45–60 ppm), O-alkyl C (60–90 ppm), di-O-alkyl C (90–110 ppm), aromatic C–C/aromatic C–H (110–140 ppm), aromatic C–O (phenols) (140–160 ppm) and carboxyl/amide C (160–190 ppm). Deconvolution of each spectrum was performed on DmFit 2011 to determine the relative intensity of each selected region (Massiot et al., 2002). The ratio of Alkyl C to O-AlkylC (A/O-A ratio) used as a sensitive index of litter decomposition extent was calculated according to Baldock et al. (1997).

2.4. Microbial basal respiration and substrate-induced respiration

Three g DW equivalent of fresh litter were placed in 117 ml glass jars. The glass jars were immediately sealed with hermetic rubber septa, and incubated for 2 h at 23 °C. Thereafter, 1 ml of air was sampled in the headspace with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to analyze the CO₂ production. The gas chromatograph was equipped with a thermal conductivity detector and a packed column (Porapack). The carrier gas helium flow was regulated at 60 ml h^{−1}. Ambient CO₂ concentrations were subtracted from sampled CO₂ concentrations and resulting values were adjusted at 22 °C according to Ideal Gas Laws using a Q₁₀ = 2. Substrate-induced respiration (SIR) rates were estimated using a procedure from Anderson and Domsch, 1978. Three grams DW equivalent of fresh litter were placed in 117 ml glass jars and amended with powdered glucose (1000 µg C g^{−1} soil) found to maximize the respiration rate in litter in a preliminary assay (data not shown). One ml of air was sampled in the head space with a syringe and injected into a gas chromatograph to analyze CO₂ production for 1 h. Substrate-induced respiration was converted into microbial biomass (MB) using the relation established by Beare et al., 1990.

2.5. Bacterial community catabolic structures

The potential of cultivable microbial communities to oxidize different C-substrates was determined with Biolog EcoPlate (Biolog, California, USA), using a procedure adapted from Garland and Mills (1991). Briefly, 4 g of dry litter was added to 100 ml of 0.1% sodium pyrophosphate sterile buffer in a 250 ml flask and shaken for 2 h (80 rpm). The litter suspension was diluted and standardized at optical density (OD) 595 nm = 0.03 with a sterile physiological solution (NaCl 0.85%). 125 µL of microbial suspension were used to inoculate each well. The plates were incubated at 25 °C for 5 days and microbial development was monitored by reading the absorbance at 590 using a TECAN® spectrophotometer. The absorbance value for each well was blanked against the control well and negative absorbance values were set to zero. The minimum OD for a positive well was fixed at 0.25 (Garland and Mills, 1991). The OD after 48 h were chosen according to the exponential phase of growth curves for all plates, for further comparison. The overall rate of substrate utilization by microorganisms was measured by calculating the Average Well Color Development (AWCD) for each plate at 48 h AWCD = Σ ODi/31 where ODi is the optical density for each well. Catabolic diversity was assessed by Shannon's diversity:

$$H' = - \sum_{i=1}^N p_i \log_{10} p_i \quad (1)$$

where p_i is the ratio of color development of the i^{th} well to the sum of color development of all positive wells.

2.6. Bacterial community genetic structure

The DNA was extracted from 0.25 g of litter, using NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer instructions and quantified using Quant-iT™ dsDNA High-Sensitivity Assay Kit (Invitrogen, Canada). Then, all DNA extracts were diluted to 0.5 ng / µL for subsequent analysis. Bacterial community structures were analyzed using T-RFLP fingerprinting as described by Bland et al. (2015). Briefly, bacterial 16S rRNA gene was amplified using primers FAM labelled 63F and 1389R. Biorad T100 thermal cycler was used for the amplification with the following programs: initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 57 °C for 45 s, and 72 °C for 90 s, followed by a final extension time at 72 °C for 10 min. Bacterial PCR products were digested with restriction enzyme *AluI* (Thermo Fisher). Restriction fragments were mixed with formamide containing 0.5% of LIZ500 internal size standard (Applied Biosystems,) and denatured at 94 °C for 3 min. Samples were electrophoresed in an ABI 3730 PRISM® capillary DNA sequencer (Applied Biosystems). The T-RFLP profiles obtained with the sequencer were analyzed using GeneMapper® v3.7 software (Applied Biosystems). The fragments between 50 and 500 bp and peaks height ≥ 50 fluorescence units were included in T-RFLP analysis. Fragments having a relative abundance of proportion $< 0.5\%$ were removed from the matrices.

2.7. Statistical analyses

The normality and homogeneity of the variances of the data were determined on the residuals from the regression model with the Shapiro-Wilk and Levene tests, respectively. Data were transformed to common logarithms, \log_{10} , when necessary to meet the requirements of normality and homogeneity of variance for ANOVA. Three-way ANOVA was used to determine whether, and to what extent, microbial properties (i.e. basal respiration, active microbial biomass via SIR, bacterial catabolic diversity index ECO H' and bacterial richness and evenness via genetic analysis) and chemical characteristics (using NMR data and C/N ratio) depended upon i) litter type, ii) the treatment (French control and Algerian control and Transfer), iii) the environmental context (coast and inland), and iiiii) their interactions. When a 3-way interaction was found, we separated data for each modality of treatment and type of litter and environmental context using a one-way ANOVA. Statistically significant ($P < 0.05$) main effects and interactions were analyzed further using the Tuckey HSD *post hoc* tests. Catabolic (Biolog®) and genetic (T-RFLP) profilings were analyzed using Principal Coordinates Analysis (PCoA) followed by Between-Class Analysis (BCA) using the litter type and the location as factors. This analysis was performed with ade4TkGUI (v.1.3) package. In addition, differences in catabolic and genetic profiles among litter types and locations were tested using a Monte Carlo test (9999 permutations). All statistical analyses were performed using XLSTAT-Pro 7.5 (Addinsoft, Paris, France) except for Biolog and TRFLP profiling analyses performed with R software (version 2.12.0 R Development Core Team, 2008).

3. Results

3.1. Pedoclimate parameters

In order to describe climate contrasts between France and Algeria across both sides of the Mediterranean, certain environmental parameters were monitored throughout the one-year incubation period in the field (i.e. April 2017-March 2018), in both coastal and inland contexts. Results recorded in the field as well as those obtained from Worldclim data (Hijmans et al., 2005) are presented in Table 1. In Algeria, maximal temperature recorded in the field reached 49 °C (July) for inland area vs 48.9 °C (August) for coastal area. In France, the maximum recorded was of 38 °C for inland area (August) vs 44.5 °C for coastal area (August). Minimal relative air humidity followed obviously opposite trends: in

France, 25.5% and 24% were recorded for inland and coastal areas respectively whereas in Algeria, values were of 5.5% in inland area vs 12.1% in coastal area. During winter, minimal temperature recorded in France was of 0 °C in coastal area vs -6.5 °C in the inland area. In Algeria, minimal temperature was of 5 °C and -5.5 °C in coastal and inland areas respectively. According to the Worldclim data (Table 1), Algeria is markedly characterized by more drastic conditions, with 15.5 °C of annual mean temperature for inland area and 18.4 °C for coastal area whereas in France the inland area is characterized by 13.1 °C of annual mean temperature and 14.1 °C for coastal area. In addition, concerning annual precipitations, they were of 350 mm and 394 mm for inland and coastal area respectively in Algeria vs 643 mm and 602 mm in France for inland and coastal areas respectively. These observations underline different tendencies according to the country: in France (Northern Mediterranean), coastal area is characterized by more arid climate conditions than inland area whereas in Algeria (Southern Mediterranean), an opposite trend was observed. Moreover, sea spray exposure was underlined in coastal areas in both France and Algeria by the higher level in both conductivity and NaCl which can be considered as a marker of sea spray considering the high proportion of such ions in sea water (Table 2): average values of 1697 and 266 $\mu\text{S}\cdot\text{cm}^{-1}$ in French and Algerian coastal areas vs 213 and 176 $\mu\text{S}\cdot\text{cm}^{-1}$ in French and Algerian inland areas, 3.05 and 0.5% in French and Algerian coastal area vs 0.4 and 0.35% in French and Algerian inland area.

3.2. Chemistry of French and Algerian litter samples at $t = 0$

Litter chemical properties characterised by solid-state ^{13}C NMR and C/N at $t = 0$ (composite sample of litter from France and Algeria used for litter bag preparation) are shown in Table 2. Whatever the country (France or Algeria) or the environmental context (coast or inland), the amount of (di-O-Alkyl C + O-Alkyl C) –referring to carbohydrates- was globally higher in *Pinus halepensis* litter compared with that of *Pistacia lentiscus*.

Moreover, NMR signals assigned to the recalcitrant fraction of organic matter were higher in *Pistacia* litter (Table 2) i.e. Alkyl C, assigned to fatty acid chains of lipids found in waxes or cutin, (aromatics + phenols) assigned to lignin and (aromatics + phenols)/N ratio, considered as an indicator of litter recalcitrance referring to lignin/N. This tendency was particularly strong in the coastal environment

3.3. Litter chemistry after 1-year of litter bag field-incubation

After the 1-year of field-incubation, ANOVA showed an interaction effect between the three factors (treatment, environmental context and litter type) on almost all the tested chemical markers (Table 3).

After 1- year of field-incubation in Algerian coastal area, transferred litter of *Pinus halepensis* and of *Pistacia lentiscus* showed lower C/N ratio compared to French control ($F = 32.198$, $p < 0.001$; $F = 217.228$, $p < 0.001$ for *Pinus* and *Pistacia* respectively). Lower (aromatics + phenols)/N ratio was observed in transferred litter of *Pinus halepensis* and of litter admixture compared to French control ($F = 9.792$, $p < 0.01$; $F = 6.071$, $p < 0.05$ for *Pinus* and admixture respectively).

Moreover, concerning Alkyl C/O-Alkyl C (A/O-A) ratio (used as a chemical index of litter decomposition extent), it was similar to French control for both monospecific litter types after transfer. On the other hand, for litter admixture, this ratio was higher after transfer (Fig. 1 (C)).

On the other hand, after 1-year of field-incubation in the Algerian inland area, transfer resulted in a lower C/N ratio for *Pistacia lentiscus* litter ($F = 477.739$, $p < 0.001$) only. (aromatics + phenols)/N ratio followed the same trend as that observed in coastal area (higher in transferred litter of *Pinus halepensis* ($F = 43.122$, $p < 0.001$) and in litter admixture ($F = 27.846$, $p < 0.001$). Concerning the (Alkyl C/O-Alkyl C (A/O-A) ratio, it was similar between transferred litter and French control for the three litter types (Fig. 2 (C)).

Table 2

Litter chemical properties of *Pinus halepensis* (Ph) and *Pistacia lentiscus* (Pl) litter collected from both contexts (inland and coast) in France and Algeria at $t = 0$ (prior to litter bags' set up): Carbon content (%C), Nitrogen content (%N), C/N, C-groups defined from ^{13}C NMR spectra, pH, Electrical conductivity (EC) and Chloride ion (%). These analyses represent a single value from a composite sample obtained from five sampling sites from either coastal or inland area, for each country respectively.

	France				Algeria			
	Inland		Coast		Inland		Coast	
	Ph	Pl	Ph	Pl	Ph	Pl	Ph	Pl
Alkyl C (Lipid)	21.99	20.48	22.79	24.95	26.56	27.7	24.79	27.91
di-O-Alkyl C + O-Alkyl C (Carbohydrate)	48.94	48.55	49.00	40.14	49.1	44.36	50.87	43.15
Methoxyl C	4.2	4.79	6.66	5.86	4.89	3.98	5.13	4.19
Carboxyl C	4.82	6.35	4.11	6.91	4.88	7.48	4.56	6.78
Aromatics + Phenols (Lignin)	20.05	19.83	17.45	22.14	14.57	9.91	14.62	17.96
(Aromatics + Phenols)/N	16.67	22.36	13.20	14.26	17.23	6.16	15.26	22.32
C (%)	46.97	43.48	48.47	49.71	51.06	47.37	50.63	43.58
N (%)	1.20	0.89	1.32	1.55	0.85	1.61	0.96	0.80
C/N	39.06	49.03	36.64	32.02	60.38	29.46	52.85	54.16
pH	6.20	7.34	6.35	6.07	6.21	6.68	6.55	7.47
EC ($\mu\text{S cm}^{-1}$)	150.2	277.3	1611	1784	104.7	249.7	225.7	306
NaCl (%)	0.3	0.5	2.9	3.2	0.2	0.5	0.4	0.6

Table 3

. Three-way ANOVA on chemical properties from solid-state ^{13}C NMR data and elementary analyses (with F and p-values) according to the three factors tested: treatment (France vs Algeria vs Transfer), environmental context (Coast vs Inland) and litter (*Pinus halepensis* (Ph), *Pistacia lentiscus* (Pl), and the equitable admixtures of Ph/Pl). ns = non-significant, * < 0.05, ** < 0.01, *** < 0.001.

Effects	d.f.	C/N		Alkyl C		di-O-Alkyl C + O-Alkyl C		Alkyl C/O-Alkyl C(A/O-A)		Aromatics + Phenols		(Aromatics + Phenols)/N		Carboxyl C		Methoxyl C	
Treatment (T)	2	11.099	***	30.607	***	1.657	ns	10.940	***	92.093	***	14.977	***	2.044	ns	1.625	ns
Context (C)	1	0.001	ns	77.807	***	10.220	**	66.405	***	9.511	**	59.815	***	23.027	***	23.415	***
Litter (L)	2	37.821	***	38.736	***	78.704	***	204.899	***	38.863	***	8.163	***	169.013	***	70.337	***
T × C	2	43.868	***	23.671	***	14.987	***	41.629	***	1.109	ns	47.706	***	0.206	ns	18.905	***
T × L	4	13.136	***	10.117	***	4.318	**	7.634	***	5.162	**	5.775	***	2.802	*	1.996	ns
C × L	2	11.370	***	1.783	ns	5.260	**	9.856	***	10.159	***	7.780	***	2.100	ns	18.098	***
T × C × L	4	104.660	***	2.670	*	4.862	**	6.687	***	5.600	***	20.584	***	1.541	ns	14.723	***

3.4. Microbial biomass and basal respiration

Results showed an interaction between the three factors (treatment, environmental context, and litter type) for microbial biomass (MB) and basal respiration (BR) (Table 4).

Figs. 3 and 4 show results for MB and BR for each context (Fig. 3 coastal area and Fig. 4 inland area) and for each treatment (transfer vs controls in France and Algeria) and litter type. For *Pinus* litter type, the same responses were observed after transfer to either coastal or inland area: MB was similar to French and Algerian controls whereas BR was lower (Fig. 3 A and B and 4 A and B). For both other litter types i.e. *Pistacia* and *Pinus/Pistacia* litter admixture, transfer to inland Algerian area led to lower MB and BR compared to both French and Algerian controls (Fig. 3 A and 4 A).

3.5. Bacterial community catabolic structure

Between Class Analyses (BCA) were performed on carbon substrate-utilization profiles of bacterial communities for each context (Fig. 5A inland area and Fig. 5B coastal area). Each BCA includes projections of the different types of litter for each country and for transfer (Fig. 5).

For each BCA (Fig. 5), the two first principal components explained 28.5% and 29.7% of the variance. The Monte Carlo test showed significant differences in catabolic structures between litter types and for both contexts.

Whatever the considered context, microbial catabolic profiles were separated in three distinct clusters according to the three different treatments (Transfer vs France vs Algeria) (Fig. 5 A and B). Moreover, for controls, catabolic structures of the three different litter types were more discriminated in France compared with Algeria whatever the environmental context, reflecting thus a more pronounced litter type effect on microbial catabolic structures in Northern Mediterranean.

ANOVA (Table 4) showed a significant effect of treatment (Transfer vs France vs Algeria) on both catabolic diversity index (Shannon-Weaver index, $\text{ECO H}'$) and Average Well Color Development (ECO AWCD). More precisely, though catabolic structures of transferred litter and of litter controls in France and Algeria were different, $\text{ECO H}'$ and ECO AWCD of Algerian litter control were lower from both other treatments (French litter control and transferred litter) ($F = 16.05$, $p < 0.0001$ for $\text{ECO H}'$ and $F = 16.12$, $p < 0.0001$ for ECO AWCD).

3.6. Bacterial community genetic structure

Between Class Analyses (BCA) were performed on T-RFLP profiles of bacterial communities for each context (coast and inland). Each BCA includes projection of the different types of litter for each country and for transfer in inland (Fig. 6 A) or coastal area (Fig. 6 B). BCA showed that 27.7% and 30.2% of the variance was explained for inland and coastal context, respectively. The Monte Carlo test showed significant differences between litter types for both contexts.

xBCA showed that whatever the context considered, bacterial genetic structures were separated in three distinct clusters according to the three different treatments (Transfer vs France vs Algeria, Fig. 6 A and B) showing that stress treatment via transfer resulted in distinct genetic structures from controls. Interestingly, bacterial genetic structures showed a clear discrimination between the three types of litter, especially for coastal context (Fig. 6 B). However, in inland area (Fig. 6 A), transfer of litter smoothed variations in genetic structures between *Pinus halepensis* and *Pinus/Pistacia* admixture. Moreover, Fig. 6A shows that the genetic structures of *Pistacia lentiscus* litter were quite similar between each other whatever their origin (France, Algeria or transfer), while those from *Pinus halepensis* (and from litter admixture) were clearly discriminated.

ANOVA (Table 4) showed a significant effect of litter type on

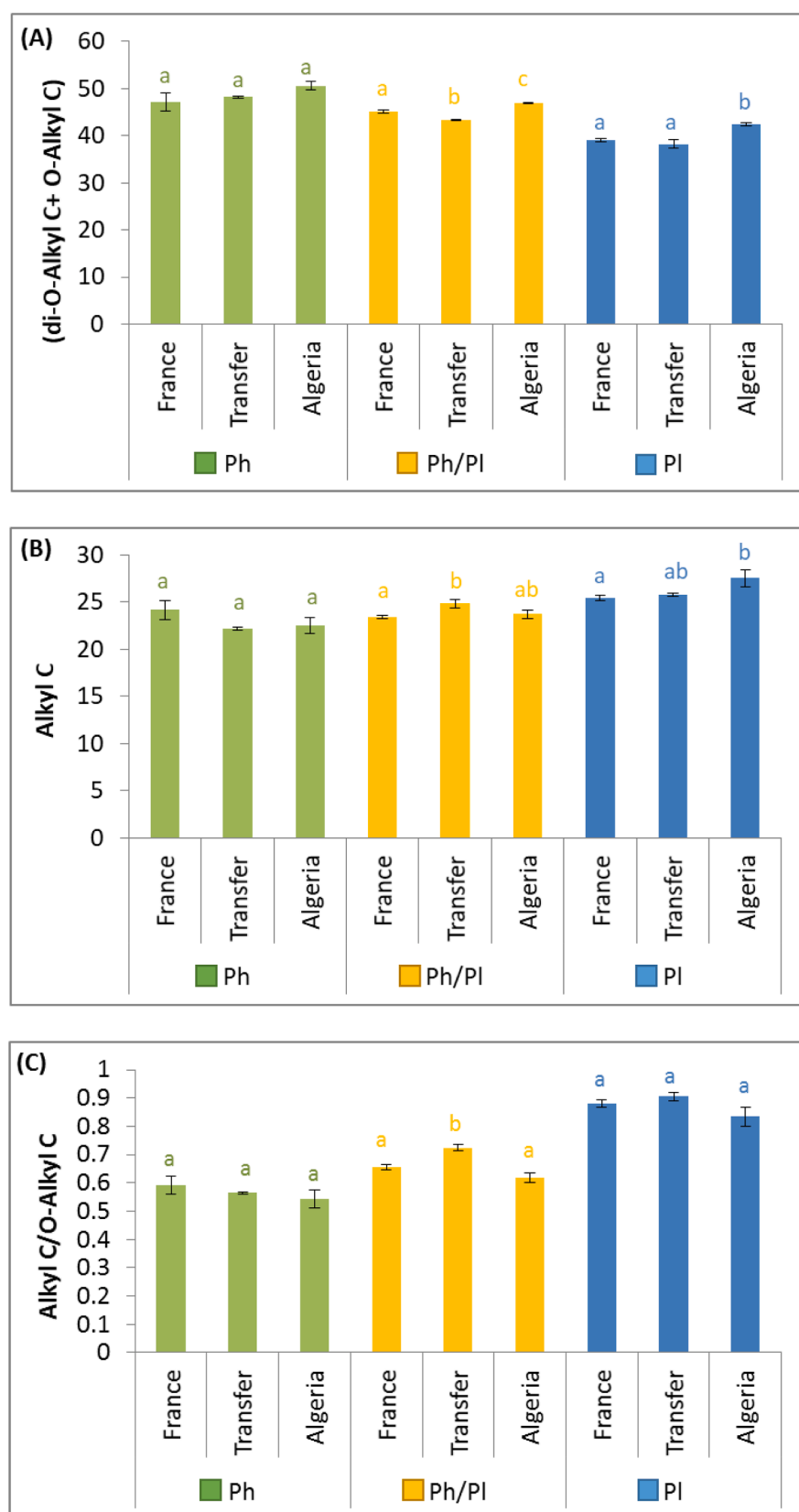


Fig. 1. (di-O-Alkyl C + O-Alkyl C) (A), Alkyl C (B), and Alkyl C/O-Alkyl C ratio (C) after 1-year of field incubation in coastal context of litter: *Pinus halepensis* (Ph), *Pistacia lentiscus* (PI) or admixture (50/50) of both litter types (Ph/PI). Transfer = Transferred litterbags, France = control litterbags in French coast, Algeria = control litterbags in Algerian coast. Letters indicate significant differences between treatment (Transfer, France and Algeria) for a litter type. Bars represent standard error. (n = 5).

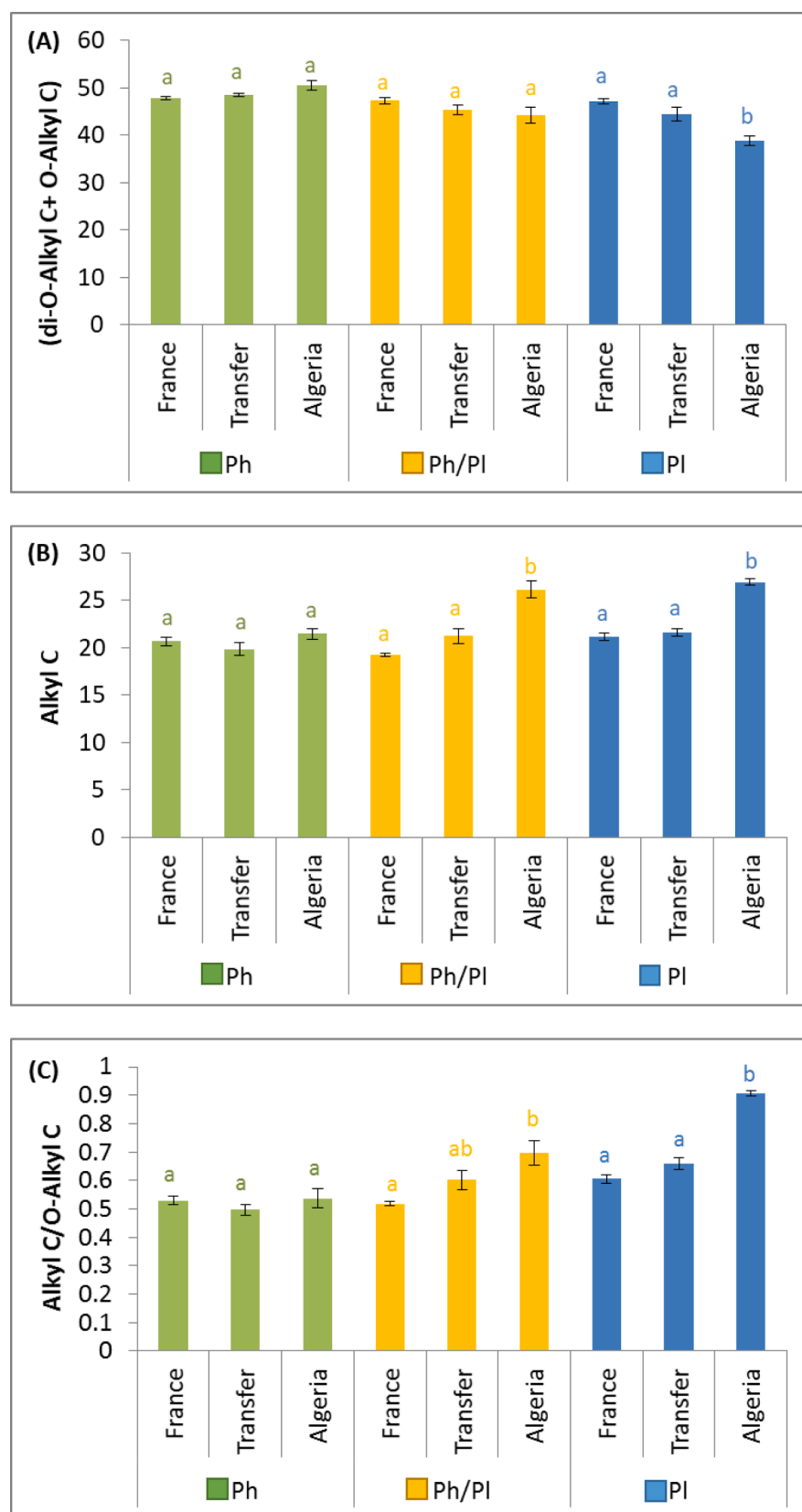


Fig. 2. (di-O-Alkyl C + O-Alkyl C) (A), Alkyl C (B), and Alkyl C/O-Alkyl C ratio (C) after 1-year of field incubation in inland context of litter: *Pinus halepensis* (Ph), *Pistacia lentiscus* (Pl) or admixture (50/50) of both litter types (Ph/Pl). Transfer = Transferred litterbags, France = control litterbags in French inland, Algeria = control litterbags in Algerian inland. Letters indicate significant differences between treatment (Transfer, France and Algeria) for a litter type. Bars represent standard error. (n = 5).

bacterial genetic richness: litter of *Pistacia* showed a higher richness than both other litter types ($F = 3.18$, $p < 0.05$). Bacterial evenness was driven by the context i.e. higher evenness for inland context ($F = 7.53$, $p > 0.01$) as well as by the treatment i.e. higher bacterial evenness in litter

from Algeria compared with both other treatments ($F = 8.65$, $p < 0.001$).

Table 4

Three-way ANOVA on microbial properties (with F and p-values) according to the three factors tested: treatment (France vs Algeria vs Transfer), environmental context (Coast vs Inland) and litter (*Pinus halepensis* (Ph), *Pistacia lentiscus* (Pl), and the equitable admixture of Ph/Pl). ECO H': Shannon-Weaver catabolic index of diversity from Biolog data, ECO AWCD: Average Well Colour Development from Biolog data, T-RF: Terminal restriction fragment. ns = non-significant, * <0.05, ** < 0.01, ***<0.001.

Effects	d.f.	Basal respiration	Microbial biomass	ECO H'	ECO AWCD	T-RF richness	T-RF evenness
Treatment (T)	2	205.611 ***	23.605 ***	19.699 ***	20.067 ***	0.769 ns	8.650 ***
Context (C)	1	2.825 ns	0.135 ns	3.564 ns	1.170 ns	0.317 ns	7.527 **
Litter (L)	2	114.373 ***	4.827 *	0.593 ns	2.944 ns	3.180 *	0.983 ns
T × C	2	159.677 ***	40.717 ***	1.207 ns	1.299 ns	0.496 ns	0.423 ns
T × L	4	25.877 ***	12.045 ***	2.138 ns	4.108 **	0.766 ns	0.260 ns
C × L	2	46.067 ***	5.052 **	2.064 ns	0.442 ns	0.776 ns	1.509 ns
T × C × L	4	12.496 ***	9.085 ***	1.945 ns	2.257 ns	0.354 ns	0.212 ns

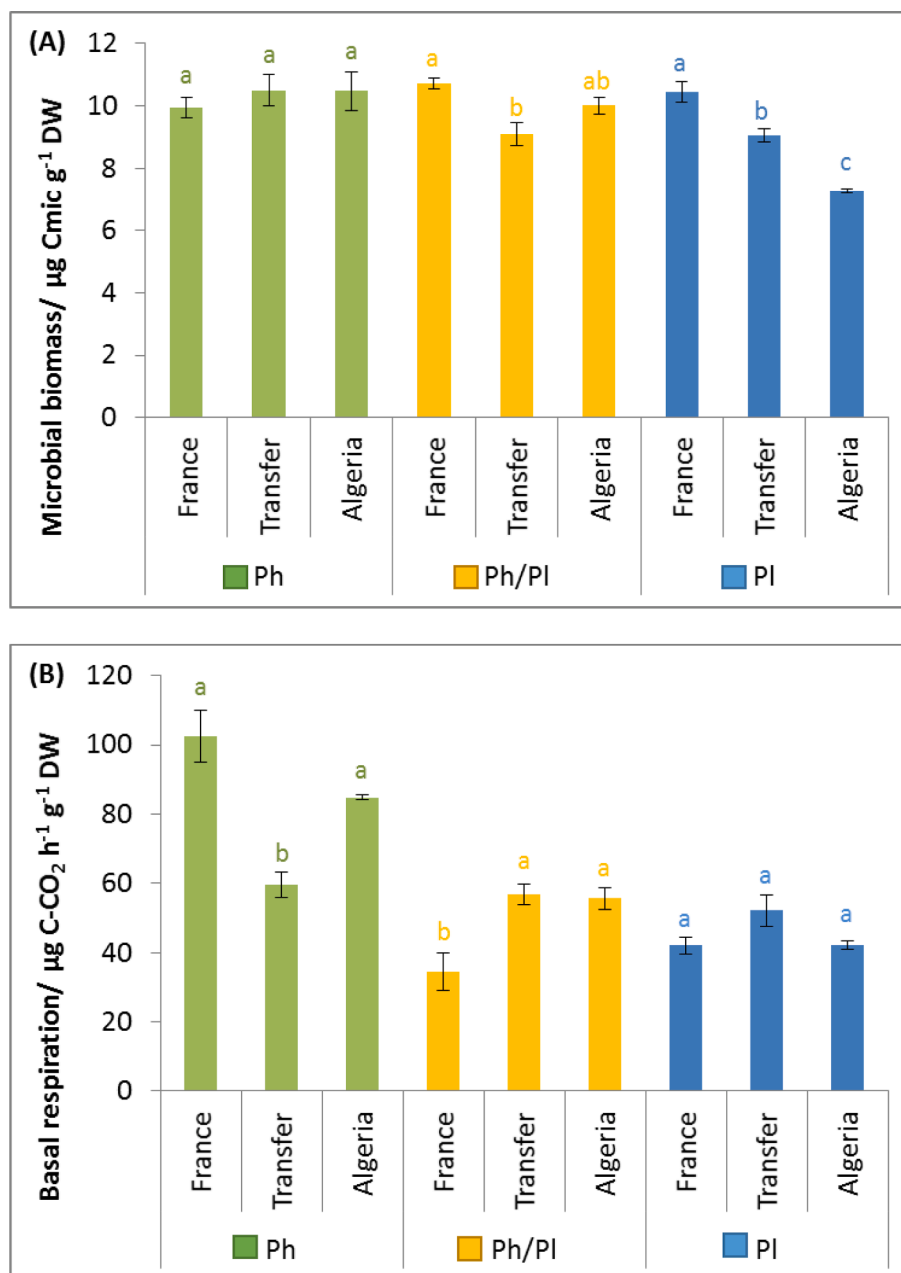


Fig. 3. Active microbial biomass (A), and basal respiration (B) after 1-year of field incubation in coastal context of litter: *Pinus halepensis* (Ph), *Pistacia lentiscus* (Pl) or admixture (50/50) of both litter types (Ph/Pl). Transfer = Transferred litterbags, France = control litterbags in French coast, Algeria = control litterbags in Algerian coast. Letters indicate significant differences between treatment (Transfer, France and Algeria) for a litter type. Bars represent standard error. (n = 5).

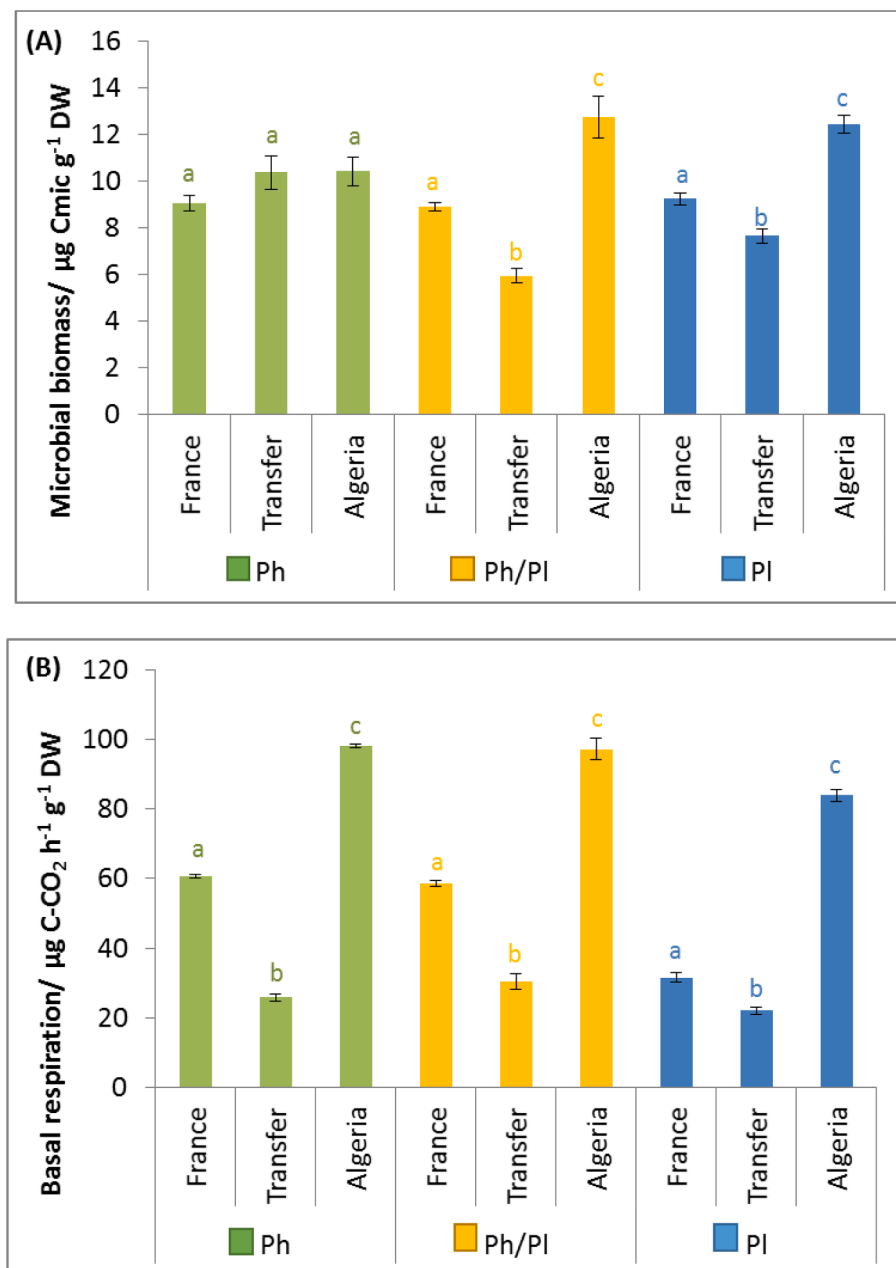


Fig. 4. Active microbial biomass (A), and basal respiration (B) after 1-year of field incubation in inland context of litter: *Pinus halepensis* (Ph), *Pistacia lentiscus* (PI) or admixture (50/50) of both litter types (Ph/PI). Transfer = Transferred litterbags, France = control litterbags in French inland, Algeria = control litterbags in Algerian inland. Letters indicate significant differences between treatment (Transfer, France and Algeria) for a litter type. Bars represent standard error. (n = 5).

4. Discussion

This study was carried out to investigate the effect of climate stress (higher temperatures and lower rainfall) on litter microbial community structure and functioning through litter transfer across the Mediterranean: from a sub-humid bioclimatic stage in France to a semi-arid bioclimatic stage in Algeria (considering both inland and coastal contexts).

After a 1-year of field incubation, we found that -whatever the context considered (coast or inland) - climate stress after transfer led to a lower microbial biomass (MB) in all litter types (relatively to French and Algerian control) except for *Pinus halepensis* litter. Zouidi et al. (2019) compared microbiological characteristics of soils from two different climate zones in Algeria (arid and semi-arid climate zones) and found lower microbial biomass and basal respiration under the more drastic

environmental conditions of the arid zone. They explained these results by climate stress characteristic of the arid zone i.e. high temperatures and long-lasting drought, which may trigger the dormancy of certain bacterial groups to withstand these stressful climate conditions. However, measurement of MB via substrate-induced respiration (SIR) technique, involved homogenized and favorable conditions of temperature and moisture, and thus the decrease in active microbial biomass cannot be attributed to dormancy but rather to microbial death (Schimel et al., 2007). It is worth mentioning that here, the climate data underlined strong climate contrasts between the four areas selected for our study. Aridity mainly refers to a deficiency of moisture (Maliva and Missimer, 2012), our observations highlighted thus increasing aridity (drier climate conditions) with decreasing latitude from the French inland area to the Algerian inland area. Since water availability is considered as the main environmental constraint in Mediterranean ecosystems (Larcher,

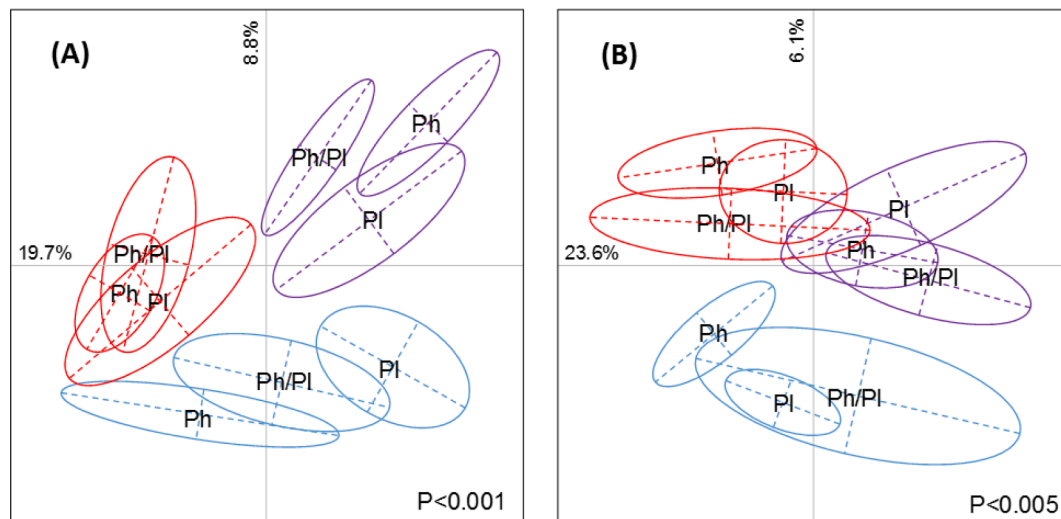


Fig. 5. Between Class Analyses representing the bacterial catabolic structures of litter: *Pinus halepensis* (Ph), *Pistacia lentiscus* (Pl) or admixture (50/50) of both litter types (Ph/Pl) in inland (A) or coastal (B) context. The blue (France), red (Algeria) and purple (Transfer) ellipses correspond to 66% of variability around the mean for the five replicates. The Monte Carlo test simulated p-value (9999 permutations) is mentioned in the lower right corner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

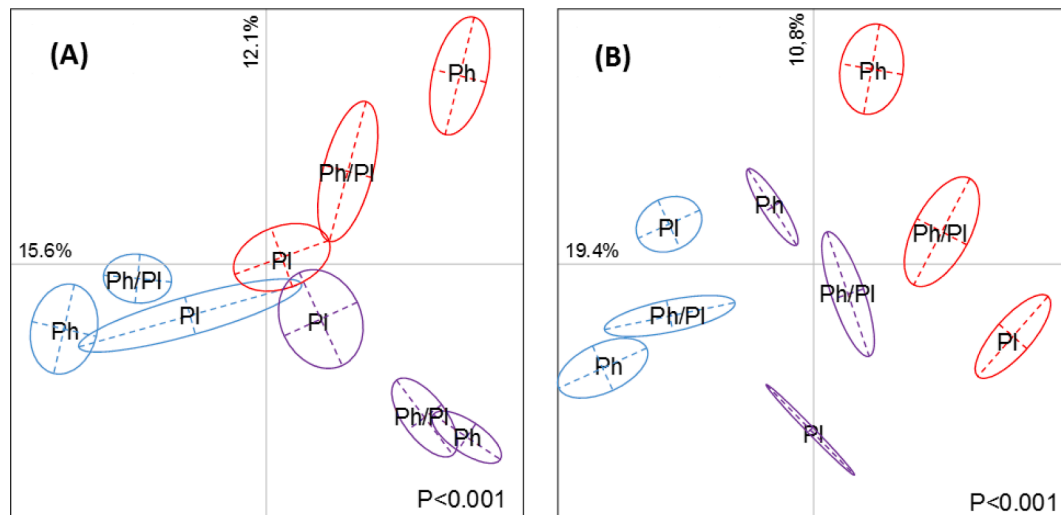


Fig. 6. Between Class Analyses representing the bacterial genetic structures of litter: *Pinus halepensis* (Ph), *Pistacia lentiscus* (Pl) or admixture (50/50) of both litter types (Ph/Pl) in inland (A) or coastal (B) context. The blue (France), red (Algeria) and purple (Transfer) ellipses correspond to 66% of variability around the mean for the five replicates. The Monte Carlo test simulated p-value (9999 permutations) is mentioned in the lower right corner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2000), “climate stress” used throughout our study refers mainly to increased aridity. Our results are thus in line with those obtained by Allison et al. (2013) and Santonja et al. (2017b), Santonja et al. (2017b) who both found a decline in litter MB in field plots subjected to drought treatment (rain exclusion/reduction) in Mediterranean ecosystems.

Furthermore, our results shed light upon the importance of litter species identity in mediating the response of microbial biomass to stress, which goes in line with the studies that highlighted the role of substrate quality and nutrient availability in sustaining microbial biomass and its stability (Karabi et al., 2015; Wardle, 1992). Here, ^{13}C NMR analyses showed that litter samples (prior to litterbag transfer) of the coniferous species, *Pinus halepensis* were chemically distinct from those of the evergreen shrub, *Pistacia lentiscus*. The latter exhibited higher amounts of recalcitrant compounds particularly in coastal area i.e. higher content in Alkyl C (fatty acids in cutin and waxes), aromatic and phenolic C (lignin) and lower content of di-O-Alkyl and O-Alkyl C (carbohydrates). A review of data obtained from ^{13}C NMR showed that higher contents in

recalcitrant compounds were found in evergreen shrub compared with coniferous species (Cepáková and Frouz, 2015). Such chemical differences may lead to distinct ecological niches and distinct selective values for microorganisms, which may differ in their sensibilities to changing climate conditions (Schimel et al., 2007; Wardle, 1992). This may explain why active microbial biomass did not decrease after transfer in *Pinus halepensis* litter only. Moreover, the distinct microbial genetic and catabolic profiles observed here for both litter types support this result. We supposed here that litter mixing would have mitigated drought stress effect on microbial communities as argued by Santonja et al. (2017b), Santonja et al. (2017b). However, here, microbial biomass from litter admixture decreased systematically after transfer in response to stress, whatever the environmental context (coastal or inland area). A decline in MB of *Pinus/Pistacia* litter admixture has been previously observed as response to drought stress induced in a laboratory experiment of Drying/re-Wetting cycles (Kheir et al., 2019).

Variations in microbial biomass after environmental stresses is of

importance since it may have considerable consequences on the global carbon cycle and more particularly on CO₂ release from soil (Wardle, 1992). Thereby, these results have to be considered together with microbial basal respiration (BR). For *Pinus halepensis* litter, after transfer, microbial communities were better able to maintain their allocation of carbon to biomass and to limit C loss through respiration (constant MB and lower BR) compared to microbial functioning of both other litter types (Schimel et al., 2007). On the other hand, microbial communities from litter admixture were indeed less efficient in allocating carbon to biomass as reflected through an increase in BR and a decrease in MB after transfer. One explanation could be that microbial communities of litter admixture contain a higher proportion of sensitive microbes to stressful conditions (after transfer in Algeria) than those of *Pinus halepensis* litter, which may have led to higher microbial mortality in litter admixture (decrease in MB). Cell lysis releases carbon and nutrients that could be used by other microbes either to support growth and biomass synthesis, or to ensure survival strategies that require high supply of carbon and energy (such as osmolyte production) or to enable concomitant degradation of recalcitrant litter compounds through a “priming effect” (Fontaine et al., 2003; Schimel et al., 2007; Van Gestel et al., 1993). All of which may trigger an increase in BR when conditions of temperature and moisture become favorable (conditions involved in BR measurement technique). This hypothesis may be confirmed by the chemical analyses conducted after the 1-year of field incubation, which revealed a higher decomposition degree in litter admixture transferred to Algerian coastal area (higher Alkyl C/O-Alkyl C (A/O-A ratio) (Bal-dock et al., 1997; Cepáková and Frouz, 2015; Li et al., 2015).

Furthermore, responses (BR and MB) of microbes from *Pistacia lentiscus* and of *Pinus/Pistacia* litter admixture were strongly impacted after transfer in Algerian inland area. Such responses are typical of non-adapted microbial communities. This is not surprising, since transfer from French inland area (the more favorable climate conditions in terms of rainfalls and temperatures) to Algerian inland area (the more drastic conditions of aridity), reflect the more drastic contrast of aridity used in this study. It has been stated that, when stress reaches a certain threshold (an extreme stress as observed here in inland context), microorganisms will be forced into dormancy (Billi and Potts, 2002) or die (Van Gestel et al., 1993). This may explain the general decrease in MB and BR observed here for both litter types. However, it is worthy of mention that only microbial communities of *Pinus halepensis* litter expressed same responses to stress (stable microbial biomass and limited CO₂ release) whatever the context of the transfer (coast or inland), showing that stress threshold of microbial communities varied according to litter type. Interestingly, these results highlight the crucial role of litter type in mediating microbial responses to stress even at large spatial scales (Northern/Southern Mediterranean).

In transferred litter, a hybrid community between microbial communities in France and Algeria, may have resulted from a partial colonization of ‘French’ litter by autochthonous microorganisms. In this case, microbial responses for instance in terms of MB and BR, would have probably followed trends in line with ‘additive effects’ (Brunel et al., 2017). Though an effect of the surrounding environment cannot be totally excluded as underlined in studies based on litterbag transfer (Perez et al., 2013), we found, after transfer, specific results in terms of functional responses (not an ‘average’ of French and Algerian controls) and which strongly depended on litter species.

When considering catabolic and genetic structures, it has to be first noted that –along the latitudinal gradient represented by the four study areas, microbial catabolic structures for control litter (i.e. Algerian and French litter placed in their ‘home sites’ and not subjected to transfer), were more homogeneous for the three litter types. On the other hand, microbial genetic structures were always distinct for the three types of litter. Such observations underline a noticeable functional redundancy among litter microbial decomposers. They also reveal that more arid climate conditions (that increased with decreasing latitude), may smooth the effect of litter type on microbial catabolic potential by acting

as a strong homogenizing force through selection of phenotypes with effective tolerance mechanisms (Curiel Yuste et al., 2014; Schimel et al., 2007). The huge print of climate conditions in structuring microbial catabolic profiles has been previously highlighted at a regional scale (Pailler et al., 2014) but also at a microlocal scale (Qasemian et al., 2014).

Furthermore, it has been suggested that alteration of microbial activities in response to stress may be related to a change in the composition of microbial communities (Schimel and Schaeffer, 2012). Here we found that both genetic and catabolic structures of litter microbial communities were modified after transfer and were different from both French and Algerian controls. We supposed that a greater impact on catabolic and/or genetic structures would be found on microbial communities transferred to Algerian inland area (more drastic arid conditions). This was true for microbial communities from *Pinus halepensis* litter and litter admixture, while for *Pistacia lentiscus*, limited modifications of bacterial genetic profiles were found after transfer to Algerian inland area. These observations indicate that the shift in catabolic and genetic structures found for *Pinus halepensis* microbial communities in response to stress, sustained microbial biomass stability and limited C losses through respiration, suggesting a better microbial physiological performance compared with those of *Pistacia lentiscus* litter. This reflects a better acclimation to stress for *Pinus halepensis* microbial communities since shifts in community composition has been suggested to be characteristic of microbial acclimation to stressful conditions (Allison et al., 2010; Walker et al., 2018). Thus, here we showed that though climate effect on microbial community structures (both catabolic and genetic) is strong, it is dependent on litter type. This shows that microbial communities and their activities are subtle indicators of soil functioning, sensitive to different environmental drivers acting at various spatial scales.

5. Conclusion

Here we found that microbial physiological responses (active microbial biomass and basal respiration, both catabolic and genetic structures of microbial communities) to more stressing climate conditions varied according to species identity (*Pinus halepensis* or *Pistacia lentiscus*) and also depending on the environmental context (inland or coastal environments). On the other hand, litter admixture (*Pinus/Pistacia* admixture) did not mitigate drought effect and further studies should include litter with different functional traits (such as broadleaved plant species) to shed further light on how diversification of resources may improve the stability of microbial functioning under stress. Deciphering how microbial communities, used as indicators of soil functioning, can cope with increased aridity is essential to understand the impact of changing climate on soil processes. Our findings here, highlight the importance of litter type at large spatial scales and that coastal environments should be also carefully taken into account when deciphering the vulnerability of ecosystems to climate change.

Author contribution

All authors contributed equally to this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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