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Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat-waves

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Abstract

In the context of Climate Change, the increasing of frequency and intensity of droughts and heat-waves constitutes a serious threat for agroecosystems in the Mediterranean region. Soils and their functions may be impacted by these extreme events through changes in the biomass, composition and activities of edaphic microbial communities. We designed an experiment to investigate changes over time in the microbial biomass, composition (EL FAME profiles) and functions (catabolic responses) after severe drought and high temperature disturbances. Impacts were assessed using indoor soil microcosms under controlled drought and high temperatures, mimicking various stress scenarios and durations in conditions of severe

drought and heat wave. Drought and heat wave restructured the soil microbial communities over the course of the experiment. This may be a consequence of inhibition and/or killing of sensitive species and selection of tolerant species by the disturbances applied, but also of the proliferation of fast growing species after environmental soil conditions had been restored. Heating dry soil at 50°C had a stronger effect than only drying. Moreover, above a critical threshold of heat wave duration, soil microbial communities may have undergone a drastic biomass killing and restructuring associated with a shift in physiological traits. In this experimental context, resilience of microbial catabolic functions was not observed and in consequence ecosystem processes such as carbon mineralization and sequestration in soil may be affected.

Key words: microbial community structure, soil, drought, heat-wave, substrate-induced respiration, resilience

1. Introduction

Global warming circulation models predict changes in spatial and temporal patterns of precipitation, including shifts in the frequency and intensity of droughts and heat-waves [1,2]. This is especially true in the Mediterranean region [3]. In August 2003, a large part of Western Europe, including France, was affected by a heat wave of exceptional duration, intensity and geographical extent. Its impacts were drastic, including the death of thousands of people, and considerable effects on ecosystems and agricultural production [4]. Extreme climatic events may be more important drivers of ecosystem functioning than mean conditions [5,4]. Soils and their functions may be impacted by these events through changes in soil temperature, water content and nutrient dynamics [6]. Soil microbial communities are highly diverse in their composition and play an essential role in nutrient cycling functions such as organic matter decomposition and mineralization,

nutrient mobilization [7]. Recent studies suggested the importance of microbial diversity for soil functioning, and if the processes that determine the rate, at which microbial services are ensured, are starting to be identified, changes in microbial communities due to environmental factors (i.e. temporal shifts in temperature and soil moisture), and their link with soil metabolic functioning should be more investigated [8]. In particular, the impact of stresses or disturbances on soil resilience (i.e. recovery over time after a stress in comparison to control) are of particular importance, in order to understand how compositional shifts changes may affect soil processes and thus ecosystem services [9].

Several studies have shown the separate impacts of soil moisture and climate warming on biogeochemical cycles, and on the composition and activities of microbial communities [10,11,12]. However, the combined effects of changes in temperature and moisture have been less investigated [13], in particular, to evidence the impact of extreme events such as severe droughts and heat waves in temperate soil ecosystems [14], in relation with their intensity and duration. If these disturbances exceed a critical threshold the community may exhibit a dramatic change in composition that would prevent activities from fully recovering [15]. In consequence, soil processes such as organic matter decomposition and nutrient cycling may be durably affected resulting in a loss of ecosystem functions and services [4].

There is a need to investigate more systematically the long-term impact of severe drought and heat wave on the soil microbial community, particularly in Mediterranean soils as they may be more affected. The aims of this study were to assess both short-term responses and changes over time of soil microbial biomass, composition and catabolic functions to various disturbances. These disturbances were studied using soil microcosms under controlled drought and high temperatures, mimicking various scenarios and durations in conditions of severe drought and heat wave. We hypothesised (1) that the effects of drying-heating on soil microbial communities may be greater than the effects of drying alone, and (2) that the

disturbance duration may increase shifts in microbial taxonomic structure decreasing recovery of catabolic functions and carbon allocation.

2. Materials and methods

2.1. Soil

The soil selected for this study was a Mediterranean agricultural cambisol. The soil-sampling site was a conventionally farmed pea field (INRA-Avignon, South Eastern France, 43° 54' 57" N and 4° 52' 58" E) and had received no chemical treatment in the month preceding soil sampling. Soil was sampled (25/03/08) from the 0-20 cm depth, with 20 soil cores (5 cm diameter) taken at random around the field and pooled (14.9% gravimetric water content). Following collection, visible soil organisms, roots and plant debris were removed and soil sample was air-dried. After drying (duration less than 24 hours), the soil was sieved to obtain homogenized 2-3 mm-sized aggregates (6.6% gravimetric water content). The soil is a fine calcareous silty clay loam with a texture of 326 g kg⁻¹ clay, 270 g kg⁻¹ silt and 33 g kg⁻¹ sand. The soil aggregates contained 11.6 g organic C kg⁻¹ soil, 1.42 g total N kg⁻¹ soil and 365 g CaCO₃ kg⁻¹ soil, and had a pH (water) value of 8.37. Water holding capacity (WHC = 31.4%) of the soil aggregates was measured as the gravimetric water content of 10 g of soil fully saturated with water and then left to stand for half an hour in a filter funnel filled with glass wool.

2.2. Experimental design

Before the experiment, soil aggregates were slowly capillary-wetted and maintained at -0.01 MPa (21.4 % water content) on a suction table for 2 weeks at ambient temperature (25°C +/- 2) and in the dark, to ensure uniform initial conditions. The soil was divided (50 g of aggregates) between 62 Petri boxes (9 cm diameter microcosms, partitioned in 14 controls and 8 microcosms per treatment). The following

treatments were then performed: (1) Control (C) soil incubated at constant conditions (-0.01 MPa, 25°C), (2) soil subjected to drying disturbance (D) (-10 MPa, 4.5 % water content) and incubated at 25°C (mimicking a severe drought alone), (3) soil subjected to drying-heating disturbance (D-H) (-10 MPa, 4.5 % water content) and incubated at 50°C (mimicking a severe drought combined to an heat wave). All treatments were conducted in darkness and at a constant temperature, with each microcosm placed in a dessicator inside an incubator. The dessicators were opened every 3 days to ensure aerobic conditions and soil water contents in the microcosms were regularly checked by gravimetric measurements (if necessary, water content was adjusted by gently spraying water into microcosms).

The total duration of the experiment from application of the stress was 84 days.

The experimental conditions were applied as follows (Fig. 1):

Microcosms of control soils (C) previously equilibrated to -0.01 MPa water potential were kept at 25°C +/-1 in a control-dessicator with a beaker containing free water to maintain high air humidity, throughout the course of the experiment (84 days). For the drying and drying-heating treatments, the soils in the microcosms were rapidly adjusted to water content close to the target water potential of -10 MPa. This was done by increasing airflow over a silica desiccant, at a temperature of 25°C. The microcosms were then divided between two drought-dessicators. The bottom of each drought-dessicator contained a saturated aqueous solution of potassium nitrate salt that controlled the air humidity, a water potential of -10 MPa was maintain in the soil microcosms [16]. One drought-dessicator was then placed in an incubator at 25°C +/-1 (treatment D) and the other in an incubator at 50°C +/-1 (treatment D-H). Three durations of treatment were then chosen: (1) Two days, which is the time scale of most drying-rewetting experiments (e.g. [17]), (2) 7 days, which corresponds to a “heat wave” for climatologists, (3) 21 days, which was the actual duration of the 2003 heat wave in the area of the sampled field (Avignon, France). After each treatment duration, treated soils were

rewetted and placed under the same conditions as those of the controls (-0.01 MPa, 25°C) until the end of the experiment.

The short-term impacts (resistance) and long term ones (resilience) after restoration of the microbial parameters in the treated soils were compared to the controls [9]. Resistance of microbial communities was evaluated 2 days after the end of disturbance. Several studies showed that resilience varies according to the type and duration of stress applied (e.g. [18,19]), and in the case of drying and rewetting stress, Fierer and Schimel [20] observed no functional resilience 6 weeks after the end of stress. We then have chosen experiment duration of 84 days (corresponding to 63 days after the end of the longest disturbance applied). Resilience of microbial communities was finally evaluated on the day corresponding to 3 times the disturbance duration after end of disturbance (i.e. 6-day resilience for 2-day treatments, which means sampling at day 8 of experiment; 21-day resilience for 7-day treatments, sampling at day 28 of experiment; and 63-day resilience for 21-days treatments, sampling at day 84 of experiment). Soils were sampled and analyzed just before and after restoration of control conditions, and at different dates until the end of the experiment: 6 to 8 g of moist soil were collected in four randomly selected microcosms from each treatment scenario (the remaining soil of the microcosm was further incubated, after weighting).

Dissolved organic carbon (DOC) analysis and substrate-induced respiration (SIR) giving community-level physiological profiling (CLPP) were performed immediately after sampling; fatty acid (EL-FAME) extractions and analysis were performed subsequently on deep-frozen soils (-25°C).

To confirm the results obtained from our experiment, it was repeated on the same soil with the higher disturbance D-H-21, compared to control. DOC and CLPP measurements were then performed at two sampling dates (23 and 84 days from start of stress) for this repeat experiment.

2.3. Soil chemical and microbial properties

2.3.1. Total dissolved organic C (DOC)

Two g of the fresh soil were extracted with 10mL of 0.5M K₂SO₄ for one hour on a shaker. K₂SO₄ extracts were filtered through 0.45 mm filters and analyzed for extractable C (Shimadzu TOC-5050A total organic C analyzer).

2.3.2 Soil respiration and Community Level Physiological Profiles (CLPP) on substrate-induced respiration

Basal (BR) and substrate-induced soil respiration (SIR) were assessed using the MicroRespTM system of Campbell et al. [21], consisting of a 96-deep-well microplate (Nunc 278012 1.2mL volume) filled with soil and addition of water only (BR) or aqueous carbon substrates (SIR), sealed individually to a colorimetric CO₂-trap microplate. Mineralization of 11 carbon substrates was tested for CLPP: four carbohydrates (glucose, sucrose, trehalose and cellulose); 3 amino acids (glycine, alanine and arginine); 3 carboxylic acids (malic acid, and 2 phenolic acids: caffeic acid and ellagic acid) and a phenol (catechol). The carbon substrates were selected for ecological relevance: Relevant to soil agro-ecosystems (i.e. plant residues, root exudates etc.), involved in desiccation tolerance (osmolytes like sucrose, trehalose and glycine [14]), and differentially mineralized. The carbon substrates were prepared in order to add 30 mg of C per mL of soil water into each deep well. Substrates that did not readily dissolve in water (caffeic acid and ellagic acid) were supplied at a concentration of 15 mg C mL⁻¹ soil water. Stock solutions of the carbon substrates were adjusted to soil pH (pH 8.4) using NaOH or HCl to avoid any substrate-pH effects on microbial communities and to minimize chemical artefacts due to carbonate-derived CO₂. The concentration of the reactants in the Cresol Red gel detector CO₂-trap microplate was the same as described by Campbell et al. [21]. Each deep-well microplate was sealed to the CO₂-trap microplate with a silicone joint (MicroRespTM, UK) and incubated in the dark at 25°C. CO₂-trap absorbance was measured at

590 nm with a spectrophotometer (HTS 7000 de Perkin Elmer) immediately before to sealing to the microplates, and after 6 h incubation. A calibration curve of absorbance versus headspace equilibrium CO₂ concentration (measured by gas chromatography) was fitted to a regression model.

The metabolic quotient qCO₂ is the ratio of basal respiration (BR) to microbial biomass, and is a sensitive ecophysiological indicator of soil stress induced by environmental conditions [22]. Microbial biomass was determined by glucose-induced respiration (GIR) [23] with MicroResp™. We divided BR by GIR to obtain an index directly correlated to qCO₂ but unitless.

2.3.3. Microbial community structure (EL-FAMES)

We used microbial fatty acids to determine microbial structure. Fatty acid profiling provides information on soil microbial biomass and community structure because phospholipids (constituents of the cell membranes) are rapidly degraded after cell death and their relative abundance differs between specific groups of microorganisms [24]. We performed the ester-linked fatty acid methyl esters (EL-FAMES) method modified from [25] Schutter and Dick 2000. Three g of soil (three replicates) were extracted with 15 ml of 0.2 M KOH in methanol (37°C, agitation, one hour). The pH of this buffer was neutralized with 2.65 ml of 1.0 M acetic acid. The EL-FAMES were extracted by adding 10 ml hexane, which was removed (5 ml) from the aqueous phase after centrifugation at 800xg for 20 min. The hexane was evaporated under a stream of N₂. EL-FAMES were resuspended in 170 µl hexane with an 30 µl internal standard (methyl stearate 0.01 M, 18:0) and analyzed by gas chromatography (GC) with an (GC Agilent 7890, USA). The GC capillary column was a medium polar cyanopropyl column (DB 23) 60 m long, with an internal diameter of 0.25mm and film thickness of 0.15µm. Flame ionization detection (FID) was performed at a temperature of 280°C using helium as a carrier gas at a flow rate of 30 ml min⁻¹. Identification of the FAMES was

performed automatically using the Agilent Chemstation software (Agilent, Inc., Palo Alto, CA) in combination with the Agilent Retention Time Locking (RTL) library for FAMES. The FAME database was extended with a mixture of 24 microbial FAMES (Bacterial Acid Methyl Esters Mix 47080-U; Supelco, Inc.) and 10-Me16:0 (Matreya). Biomarkers of specific functional groups were assigned according to classification of Frostegård et al. [25]. Iso- and anteiso-fatty acids were taken as Gram-positive (G+) bacterial biomarkers (i15:0, a15:0, i16:0, i17:0), and mono-unsaturated and cyclopropyl fatty acids (16:1 ω 7c, 17:0 cy, 18:1 ω 7c, 19:0 cy) as Gram negative (G-) bacterial biomarkers [26]. The FAMES 18:2 ω 6c and 10Me16:0 were used as indicators for the fungi (FUNG) and actinobacteria (ACT) populations respectively [26,24]. The sum of all FAMES (nmol g⁻¹ dry soil) was used as an estimate of microbial biomass. The ratio of saturated to unsaturated fatty acids (SAT/UNSAT) was used as a stress indicator [27]. FAMES that occurred as less than 5% of all samples were excluded from the data set.

2.4. Data analysis

The values of the microbial parameters (BR, GIR, qCO₂, EL-FAME biomarkers) and DOC for the different treated soils were normalized to the control values % change from control [28]. To compare treatments and disturbance duration impacts on microbial parameters and DOC we applied non-parametric statistical procedures ([29]. Mann-Whitney tests were performed on raw data to assess the difference between the control and treated microcosms; Kruskal-Wallis tests and post hoc paired comparisons tests (Bonferroni) were performed on % change from control to assess the difference between disturbance durations (n = 4 for SIR measurements, BR and qCO₂, n = 3 for DOC and EL-FAMES measurements, the significance level was set to 5%). Non-parametric redundancy discriminant analysis (RDA), followed by a Monte Carlo permutation test (5000 random permutations; CANOCO 4.5 software; [30]) were used to test the statistical significance of the explanatory variables (factors): disturbance

treatment-type, disturbance duration and recovery time (i.e. time elapsed between end of disturbance and time of measurement), and the interactions between these factors on CLPPs and EL-FAME data. The EL-FAMEs structural differences and CLPP functional community differences between treated and control soils were described using the Bray-Curtis dissimilarity index (BCI, [31]).

3. Results

Mean values of biotic parameters and DOC of control soil are listed in the Table 1.

3.1. Soil respiration, metabolic $q\text{CO}_2$ and DOC

Soil-microcosms subjected to disturbance lasting 21 days, whether drying (D) or drying-heating disturbance (D-H), showed changes in basal respiration (BR). Increased respiration was detected 2 days after the end of the disturbance (Tab.2), with a maximum value of $0.88 \mu\text{g CO}_2\text{-C dw.g}^{-1} \text{ h}^{-1}$ for the D-H-21 disturbance treatment (compared to $0.25 \mu\text{g CO}_2\text{-C dw.g}^{-1} \text{ h}^{-1}$ for the control at day 23, Tab.1). Basal respiration recovered to control values at resilience-dates ($0.60 \mu\text{g CO}_2\text{-C dw.g}^{-1} \text{ h}^{-1}$ at day 84, Tab.1) for soil-microcosms subjected to drying. For soil-microcosms subjected to a drying-heating disturbance no resilience was apparent at the end of experiment and D-H soil-microcosms showed a decrease in BR compared to the control (Mann-Whitney tests; $p \leq 0.05$, $n=4$) (Tab.2). Except for the 7 days disturbance duration, disturbed soil showed a significant increase in metabolic $q\text{CO}_2$ two days after the end of the disturbance (Mann-Whitney test; $p \leq 0.05$; $n=4$) (Fig. 2. A-B), especially for the 21 days drying-heating disturbance (D-H-21). After that, metabolic $q\text{CO}_2$ decreased to control values over the course of the experiment, but remained significantly higher for soil subjected to D-H-21 disturbance treatment (Mann-Whitney tests; $p \leq 0.05$; $n=4$). DOC presented high concentrations in soil two days after the end of

disturbance (with a maximum of $136.7 \mu\text{g C dw.g}^{-1}$) and no recovery for D-H-21 disturbance treatment (Mann-Whitney tests; $p \leq 0.05$; $n=3$) (Fig. 2. C-D).

Except for the resilience in $q\text{CO}_2$ and DOC parameters of drying-disturbed soils, disturbance durations (2, 7 and 21 days) have significant effects on % change relative to control of BR, $q\text{CO}_2$ (Kruskal-Wallis tests; $p \leq 0.05$; $n=4$) and DOC (Kruskal-Wallis tests; $p \leq 0.05$; $n=3$) in term of both resistance and resilience (Tab.2, Fig.2). Generally, the longer the disturbance duration, the greater the impact (especially with high-temperature disturbance on Basal Respiration, Post hoc paired comparisons test $p \leq 0.05$). However, this pattern was not straightforward. In some cases, 2-day disturbances had a stronger impact than 7-day disturbances (i.e. $q\text{CO}_2$ of drying-heating-disturbed soils, Post hoc paired comparisons test $p \leq 0.05$) (Fig.2).

3.2. Community Level Physiological Profiles (CLPPs)

The redundancy analyses (RDA) performed on the MicroRespTM profiles (CLPPs) showed that disturbance treatments (D and D-H), disturbance durations (2, 7 and 21 days) and post-disturbance recovery time, all had significant effects on the catabolic functions of the soil microbial communities (Fig. 3). Moreover, Figure 3-A shows that disturbance duration interacts significantly with the type of disturbance treatment: the longer the disturbance duration the greater the changes in CLPPs, especially for dried-heated soils (D-H). Figure 3-B shows that the differences between CLPPs in disturbed soils increase with post-disturbance recovery time: Controls (C) differ sharply from the dried-heated soils (D-H), with the dried soils (D) occupying an intermediate position on the factorial map. In terms of resistance and resilience, the Bray and Curtis dissimilarity indices (BCI) confirm the RDA analyses. The effects of disturbance duration on CLPP using BCI index were significant (Kruskal-Wallis tests, $p \leq 0.05$, $n=4$), and the effects of disturbance treatment type were also significant for the 7-day and 21-day disturbances (Tab.2, Mann-Whitney tests, $p \leq 0.05$, $n=4$).

Soil respiration on osmolyte substrates (such as sucrose, trehalose and glycine) were not differentially affected by the disturbances, compared to other substrate-induced respirations (data not shown).

3.3. Microbial community structure (ELFAMEs)

The redundancy analysis (RDA) performed on the EL-FAMEs shows that disturbance treatment interacts significantly with disturbance duration (7 and 21 days) (Fig 4-A): the disturbance duration effect was particularly strong with the soils subjected to D-H disturbance. Figure 4-B- shows that disturbance treatment also interacts significantly with post-disturbance recovery time. And this was confirmed by the BCI performed on EL-FAMEs: the effects of the disturbance treatments on EL-FAMEs using BCI index were only significant for the 21-day disturbances (Tab. 2, Mann-Whitney tests, $p \leq 0.05$, $n=3$). The effects of D disturbances on the EL-FAME biomarkers differed from the effects of D-H disturbances. The difference was particularly marked for the longest disturbance duration (21 days, Fig. 5). More precisely, the D-21 disturbance, just before rewetting the soil, had no significant effect on the EL-FAME biomarkers (Fig. 5-A) except for G+ indicators, which increased compared to the control (18.1% +/- 3.9). The short-term impact of drying/rewetting (two days after rewetting at end of disturbance) was a decrease in EL-FAME biomarkers (Fig. 6-A), except for G+ indicators. Almost all EL-FAME biomarkers of D-21 soil had returned to the level of the control soil by day 22 after the end of the drying disturbance and remained at that level until the end of the experiment. The one exception was ACT, which decreased (-20.0% of control +/- 4.7, day 84). The D-H-21 disturbance, just before rewetting of the soil, had various effects on the different EL-FAME biomarkers (Fig. 5-B). At this stage, there was a strong decrease for FUNG (-72.8% +/- 26.5) and an increase for G+ indicators (17.3% +/- 2.9) compared to the control. Two days after rewetting and lowering the temperature, the short-term impact of the D-H disturbance was a stronger decrease of G+ indicators and ACT,

and a weaker one for G- indicators and FUNG. All EL-FAME biomarkers in the D-H-21 soil were lower than the control values. Over the course of the experiment some inhibited microbial groups were stable (G- and FUNG) while others showed increased inhibition (G+ and ACT). At day 84, ACT had completely disappeared (n = 3). No EL-FAME biomarkers in the D-H-21 soil remained at the same level as in the control soil.

The saturated/unsaturated fatty acids ratio was raised by drought and heat wave disturbances (Table 2), especially for the 21-day disturbances (at day 63 the SAT/UNSAT ratio of the D-H-21 soils was still high: 20.42). At the end of experiment the collapse (with a high variability) of the SAT/UNSAT ratio of the D-H-21 soil was due to the collapse of all the microbial biomass at this date (confirmed by total EL-FAMES and SIR, Tab. 2).

4. Discussion

4.1. Consequences of severe drought and high temperature on both short and long-term responses of soil microbial communities

The stimulation of the basal respiration two days after end of disturbance suggests an increase in C substrate availability, which is often a limiting factor for both microbial growth and activities, regardless the disturbance regime. The concomitant increase in DOC amounts confirms this hypothesis. This process called “Birch-effect” [32] can be attributed to two mechanisms [33]. One is the “substrate supply” mechanism; a physical disruption of soil aggregates due to drying-rewetting cycle (and probably also heat; [34]), which releases soil organic matter making it available to microorganisms [35,17]. The other is the “microbial stress” mechanism; releases of two sources of microbial carbon after disturbance: (i) from dead and lysed microbes due to drought, heat and rewetting [28], and (ii) from osmolyte intracellular compounds synthesized during drying stress [17]. DOC from lysed microbes is confirmed by an important decrease in microbial biomass (SIR Glucose and total EL-FAMES)

for most of disturbance regimes. We were not able to show via our MicroRespTM measurements on specific C substrates, if some drought-disturbed microbial communities would be specifically adapted to mineralizing osmolyte substrates. Therefore in our case, the microbial DOC mineralized after the disturbance may result mainly from cell death instead of osmolyte release [32]. For the 2 and 7 days disturbance durations, the “Birch-effect” showed two days after rewetting was slight, probably because of a beginning of resilience of the pulses of CO₂ and DOC after rewetting [17] in this Mediterranean soil adapted to frequent wet/dry cycles.

At long term, a 21 days duration heat wave, in contrast to drought, dramatically impacted microbial biomass (total EL-FAMES amounts and SIR) suggesting a lasting overall impact [36]. As we have seen, metabolic qCO₂ recovered to control values after the disturbance in drought soils but remained high until the end of the experiment in drought-heated soils. At short-term, the high values of this BR to biomass ratio are a consequence of direct killing biomass induced by both disturbance regimes. The qCO₂ is also known to increase when microbial communities divert a higher proportion of carbon source to maintenance-energy requirements than to biosynthesis as a result of an exogenous disturbance [22]. The physiological costs imposed on soil microorganisms [17], as well as direct killing of microbial biomass especially by heat wave disturbances, may be great enough to cause at long term shifts in the allocation and fate of carbon in the entire microbial community.

EL-FAME community structure measurements differentiated between the impacts of drought and heat wave on the composition of the microbial community, with an intermediate effect and resilience for soils subjected to drought, in contrast to soils subjected to heat wave, which did not present resilience. Moreover, changes in CLPP also suggest differences of impact in functional structure, concomitant to taxonomic shifts.

The decrease in the concentration of microbial EL-FAME biomarkers in treated D-21 soil two

days after restoration reveals the impact of an osmotic shock of re-wetting induced by rewetting rather than a direct drying disturbance impact [12]. The exception of G+ organisms, which were not inhibited by drying or by rewetting, may be explained by their thicker cell walls, giving capacities of adaptation to changes in water potential [37]. Fungi, as well as Actinobacteria, are known to be tolerant to drying [37], but in our study they were affected by drying-rewetting because of their sensitivity to osmotic shock of rewetting [38,12].

In the context of heat wave disturbance the restoration of environmental soil conditions (rewetting and lowering temperature) also impacted drastically on microbial community composition. G+ biomarkers were not impacted by the combination of high temperature and drought (as shown at day 21). G+ i17 :0, which was the major EL-FAME biomarker of the G+ group, indicates a bacterial group known to be thermophilic [27]. However, in contrast to their behaviour in the dried-rewetted microcosms, in D-H microcosms the G+ group presented sensitivity to rewetting and lowering temperature (from day 21 to day 23). Actinobacteria (known to be sensitive to high temperatures; [27]) were dramatically impacted by heat wave. The 50°C temperature disturbance combined with the osmotic shock of rewetting probably impacted irreversibly on the biomass of this known K-strategist group. Fungi (also known to be sensitive to high temperatures; [27]) were strongly impacted by heat wave disturbance; if the restoration of environmental soil conditions did not induce further inhibition of this microbial eukaryote group, we did not measure any resilience. In a recent study on recovery of soil microbial community subjected to high temperatures, Barcelá-Moreno and Bååth [39] showed that heating of soil shifted the recolonizing microbial community from fungi towards bacteria. The G- EL-FAME biomarkers were impacted by heat wave disturbance but stayed at the same concentration levels throughout the experiment after restoration; they were thus the least inhibited group at the end of the experiment. Gram-negative bacteria like Proteobacteria are r-strategists (or copiotrophic, [40]) and their ability

to grow fast may have favoured their biomass stability, or even growth, as shown in the D-21-treated soil at the end of experiment. Similarly, Thompson et al. [41] observed high CO₂-C efflux correlated with the development of Proteobacteria in soils previously dried and rewetted.

The increases in ratio of saturated to unsaturated fatty acids we measured in disturbed soils may be attributed to rapid physiological adjustments by certain microorganisms and especially to higher survival rates in drought and high temperature tolerant species compared to others [27].

However, one must bear in mind that the EL-FAME extraction method presents some limitations for characterizing microbial communities in environmental samples (contaminations with no microbial FAMES, [24]). And because of possible conservation of FAMES on dead microbial cells in dry environmental conditions, this may have led us to underestimate the impact of desiccation on the microorganisms [28].

To sum up, the restructuring of microbial communities during the course of the experiment after the end of heat wave disturbance may be a consequence of drastic impact on K strategic sensitive species (i.e. Fungi and Actinobacteria), selection of tolerant species (i.e. G + bacteria), and the development of fast-growing copiotrophic species (i.e. G - bacteria) after the restoration of environmental soil conditions, stimulated by soil organic matter that had become available from killing biomass and lack of competition.

Figure 6 presents a schematic representation of the soil microbial taxonomic and functional responses to the heat wave (Fig. 6A) and drought (Fig. 6B) 21-day disturbances in the context of our experiment. Drought and rewetting is characterized by a “Birch effect” and resilience occurring about 30 days after the end of disturbance. This recovery suggests the low impact of a severe drought on the physiological traits of this Mediterranean soil, which *in situ* was frequently exposed to moisture stress. Moreover we have to take into account the soil air-

drying and rewetting pre-treatment that may have induced microbial community selection [42] and a previous “Birch effect” [17]. By contrast, heat-drought disturbance combined with shock of rewetting during restoration conditions induced a drastic initial biomass death and increased shifts in taxonomic and catabolic structure and carbon allocation until the end of our experiment with no resilience.

4.2. Consequences of the different disturbance durations for microbial communities

Increasing disturbance duration have induced an increased release of available organic matter (as measured in DOC after the end of disturbance) and favoured C mineralization, as we observed increase in basal respiration two days after the end of the disturbance.

Resilience of microbial parameters was inversely related to duration of disturbance. This was particularly true for heat wave disturbances because, as we described, no resilience was observed in D-H-21 disturbed soils. Indeed in these soils a drastic restructuring of microbial communities and a collapse of microbial biomass were observed during the course of the experiment, whereas microbial resilience was observed in the D-H-7 disturbed soils. This study suggests that drought and heat wave induced gradients of reduction in microbial biomass and selection pressure related to the duration of disturbance (Fig. 6C). Banning and Murphy [43] similarly observed that resilience of soil SIR to heat disturbance was positively related to the size of the initial microbial biomass. Our results further suggest that there was a critical threshold of heat wave duration (somewhere between 7 and 21 days) above which no resilience of microbial parameters was observed.

In some cases the 2-day disturbed soils presented stronger reactions in their microbial communities than the 7-day disturbed soils. This may be because less than two days of desiccation directly followed by watering was not sufficient to increase the proportion of inactivated or tolerant microorganisms (dormant state or spores) by starvation [35] and/or to induce an adaptation of microorganisms at the colony scale (e.g. biosynthesis of protective

EPS by microcolonies, [44]). Such inactive, tolerant and adapted microorganisms may be less susceptible to the effects of rewetting disturbance [12].

Disturbance treatments and their duration were better differentiated during the course of the experiment and at the end (resilience) than just after the end of the disturbance. Kuan et al. [19] and Philippot et al. [45] observed, in studies respectively addressing the carbon and nitrogen cycles, that resilience of microbial parameters could be an indicator of pre-exposure to disturbance, but resistance was less. In some cases (especially the 21-day heat wave treatments) we observed no resilience 63 days after restoration of environmental conditions. Moreover, the disturbance seemed to have a lasting effect on some microbial parameters that were dramatically altered by the end of experiment. Soil microbial communities may have then undergone a drastic biomass killing inducing a shift in composition and a shift in physiological traits (e.g. allocation of carbon demand for energy requirements versus growth) as revealed by total EL-FAMES, SIR and qCO₂ measurements (Fig. 6). As a result, no resilience seems to be possible [15].

In conclusion, these results represent a contribution to the understanding of soil microbial community dynamics in the context of Mediterranean heat-wave disturbance in terms of loss of microbial biomass, shifts in community structure and carbon allocation [4]. However, our study is limited to one soil (although representative of Mediterranean agricultural soils) and our experimental conditions do not allow us to draw conclusions about the long-term dynamics of changes in soil microbial communities beyond 63 days after the end of the longest disturbance. We must take into account that our microcosm experiments probably induced a confinement effect and no possibility of microbial re-colonization from unperturbed soil [46], interaction with plant growth and recovery of soil carbon supply [47], which could contribute to some recovery of microbial biomass and functions of our high-disturbed soils. A

next step for studying heat wave impacts on soil microbial communities will be to introduce experimentally the possibility of re-colonization from the unperturbed soil. Moreover to conclude forward about the specific effect of high temperature into a drought combined to heat disturbance, further experiments should assess the effects of high temperature with and without drought on soil microbial communities.

We also need further detailed analyses in terms of community diversity (e.g. complementary FAMES biomarkers as mycorrhizal indicators, biomolecular analysis as qPCR targeting microbial groups) and functionality (e.g. bacterial and fungal contributions in carbon allocation, [39]; soil enzyme activities targeting the C and N cycles, [11]). Incorporating this knowledge into models would enhance our ability to predict soil ecosystem responses to global climate change [15,13].

5. Acknowledgements

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1 Figure 1: A schematic diagram (not to scale), illustrating the time course of drying
2 stressdisturbance and drying-heating stressdisturbance (simulating drought and heat wave events
3 respectively) and the soil water potentials during the experiment.: At day 0 of the experiment, the
4 stressdisturbance is applied. Diamonds represent sampling dates, triangles represent dates chosen
5 to represent short-term impacts resistance and scoares dates chosen to represent resilience.
6

7 Figure 2: Percentage change relative to control soils for qCO₂ (Fig. 2A, Fig. 2B) and for DOC
8 (Fig. 2C, Fig. 2D), during the course of experiment (crosses indicate values from repeated
9 experiment,, day 23 DOC analyseis were lost). Vertical bars indicate the standard deviation (n=4
10 for qCO₂ and n=3 for DOC values).

11 Stars (*) indicate significant difference between the control and treated microcosms (Mann-
12 Whitney tests performed on the parameter raw data, $p \leq 0.05$).
13

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Automatique

14 Figure 3: Factorial maps and factors effects (F values and probability, * for $p < 0.05$ and ** for
15 $p < 0.01$) from redundancy analyses performed on CLPPs., Fig. 3A and considering as source of
16 variation as stressdisturbance treatment (C=control; D-25= Drying stressdisturbance; D-50H=
17 Drying- heating stressdisturbance) and disturbance duration (2; 7; and 21 days). Fig. 3B
18 considering source of variation as disturbance treatment and recovery time considered after the
19 disturbance (days 0 to 84 after the start of the disturbances). Each triangle and circle is the
20 barycentre coordinate from replicates of each perturbation treatment, perturbation duration or
21 recovery time.(Fig. 3A and Fig. 3B, representss the correlation plot between the RDA axes and
22 the carbon sources selected for SIR) or stress treatment and the recovery time considered after the
23 stress (Fig. 3C and Fig. 3D, represents the correlation plot between the RDA axes and the carbon
24 sources selected for SIR). [Pas très clair. Diviser en deux ou trios phrases?]

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Mis en forme : Anglais (États Unis)

Figure 4: Percentage change relative to control soils for respiration induced by substrate chemical groups induced respiration. Treatments: 21-day dDrying 21 days duration stress treatment (D-25-21, Fig. 4A), drying-heating 21- day drying-heating s duration stress treatment (D-50-21, Fig. 4B). Open White symbols indicate values of the repeated experiment (Fig. 4B). Vertical bars indicate the standard deviation (n=4).

Figure 54: Factorial maps and factor effects (F values and probability, * for $p < 0.05$ and ** for $p < 0.01$) from redundancy analyses performed on EL-FAMEs. Fig. 4A considering source of variation as disturbance treatment (C=control; D= Drying disturbance; D-H= Drying-heating disturbance) and disturbance duration (7; and 21 days). Fig. 5B considering source of variation as disturbance treatment and recovery time considered after the disturbance (days 0 to 84 after the start of the disturbances). Each triangle and circle is the barycentre coordinate from replicates of each perturbation treatment, perturbation duration or recovery time.

Factorial maps and factors effects (F values and probability, * for $p < 0.05$ and ** for $p < 0.01$) from redundancy analyses performed on FAMEs, and considering as source of variation as stress treatment and duration (Fig. 5A and Fig. 5B, represents the correlation plot between the RDA axes and the FAMEs identified for more than 5%) or stress treatment and the evolution time considered after the stress (Fig. 5C and Fig. 5D, represents the correlation plot between the RDA axes and the FAMEs identified for more than 5%). C=control; D-25= Drying stress; D-50= Drying heating stress (7 and 21 days duration treatments). [En faire plusieurs phrases?]

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48 Figure 65: Percentage change relative to control soils microbial EL-FAME biomarkers.
 49 Treatments: Drying 21-day drying days duration stressdisturbance treatment (D-25-21, Fig.
 50 6A5A), drying-heating 21-day drying-heating days duration stressdisturbance treatment (D-50H-
 51 21, Fig. 56B). Vertical bars indicate the the standard deviation (n=3).

53 Figure 76: Schematic representation of the soil microbial community response to 21-day the heat
 54 -wave stressdisturbance (Fig. 7A6A) and drought stressdisturbance (Fig. 7B6B) 21 days stresses.
 55 Illustration of the impacts (measured at “resilience dates”) of stressdisturbance duration on soil
 56 samples subjected mitted to heat wave (Fig. 7C6C). Resilience dates: day 8 for the 2 day-
 57 disturbance; day 28 for the 7 day-disturbance; day 84 for the 21 day-disturbance.

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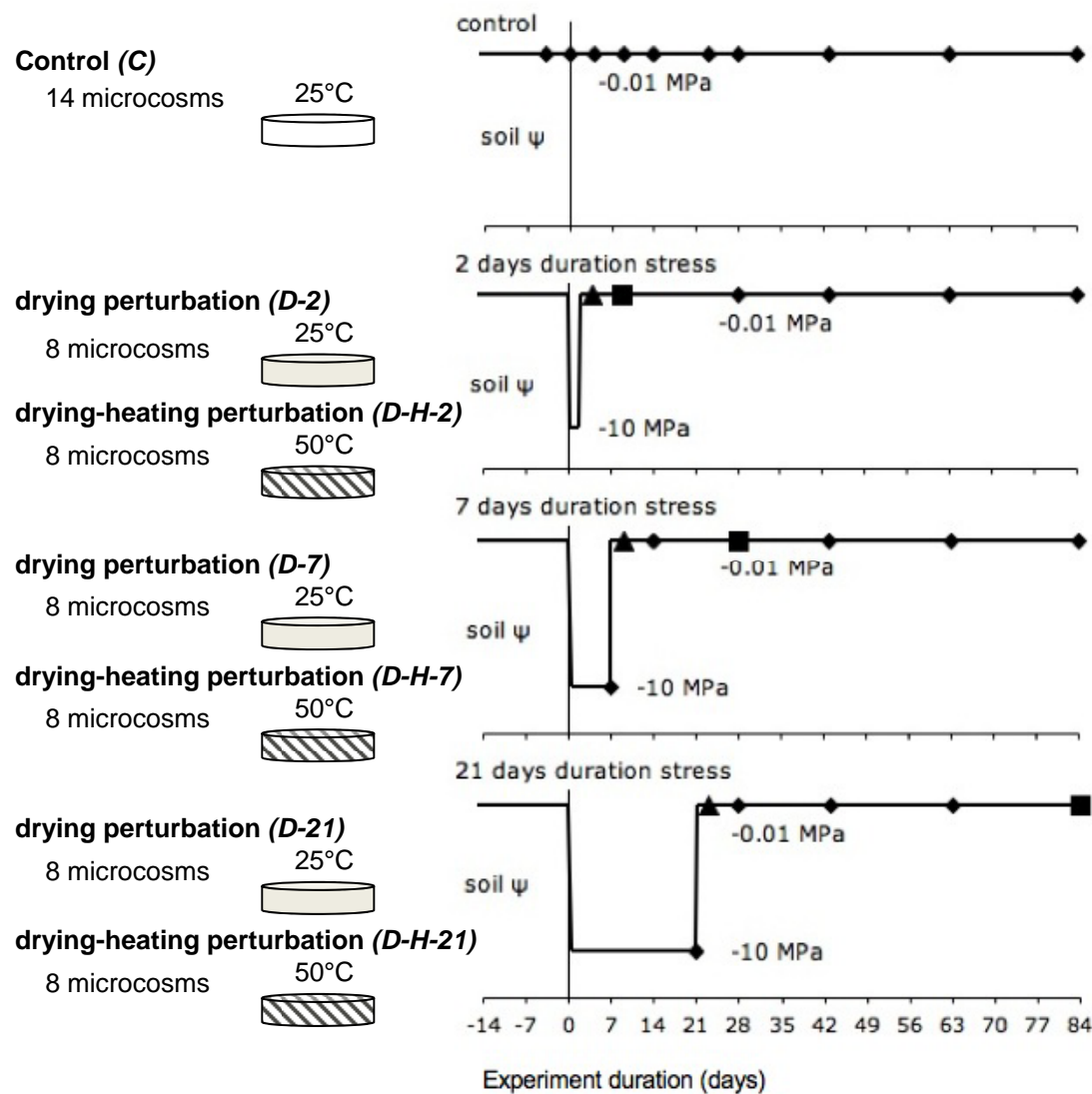
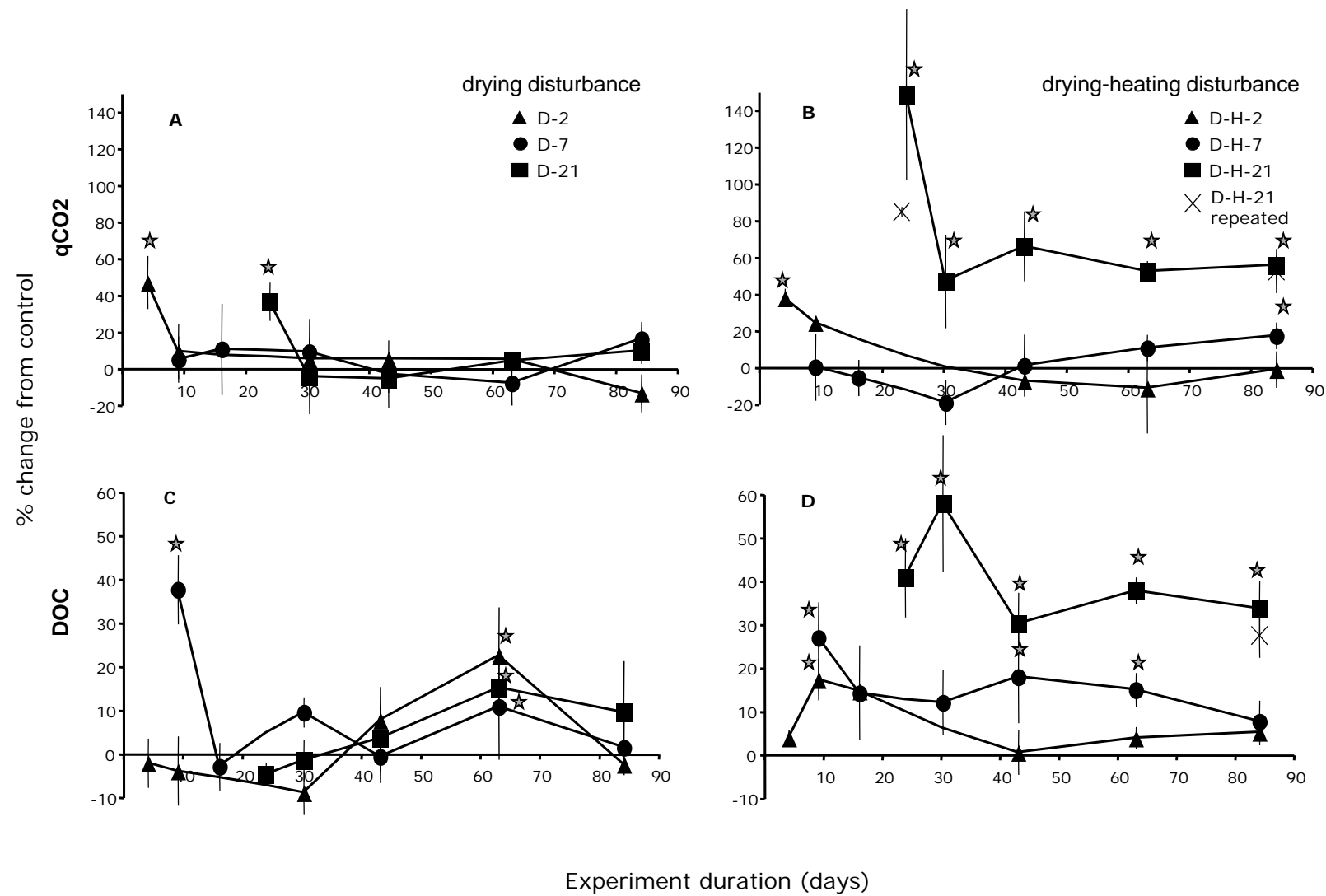


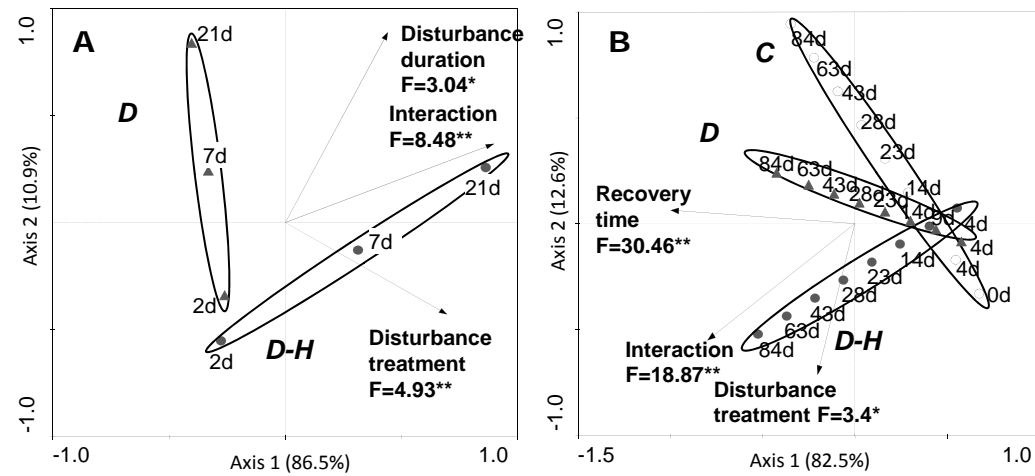
Table 1: Mean values of biotic parameters and DOC of control soil, measured during the experiment (standard deviation in brackets; n=3 for ELFAMEs and DOC, n=4 for respiration, SIR measurements and qCO₂). FAMEs biomarkers of specific functional groups: Actinobacteria (ACT); Fungi (FUNG); Gram-positive bacteria (G+), Gram negative bacteria (G-).

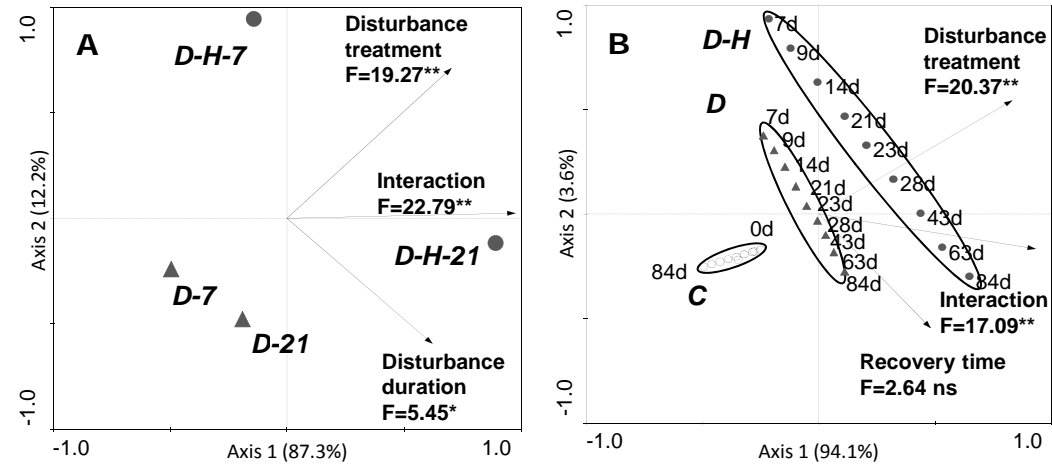
Days of experiment	0	4	9	21	23	28	84
Basal respiration (µgC-CO ₂ g ⁻¹ dry soil h ⁻¹)	0.84 (0.19)	0.72 (0.02)	0.77 (0.07)		0.25 (0.03)	0.26 (0.04)	0.60 (0.00)
SIR Glucose (GIR) (µgC-CO ₂ g ⁻¹ dry soil h ⁻¹)	1.07 (0.13)	1.39 (0.17)	1.21 (0.42)		0.76 (0.12)	0.68 (0.20)	1.41 (0.08)
qCO ₂	0.79 (0.14)	0.53 (0.08)	0.68 (0.19)		0.33 (0.07)	0.39 (0.06)	0.43 (0.02)
DOC (µgC g ⁻¹ dry soil)	85.75 (0.75)	83.05 (9.47)	68.75 (2.26)		96.98 (0.31)	86.56 (6.96)	95.14 (6.55)
Total FAMEs (nmol g ⁻¹ dry soil)	63.44 (20.84)	62.85 (2.97)	68.89 (5.46)	50.29 (25.08)	66.65 (6.43)	69.62 (15.57)	53.36 (4.71)
ACT (nmol g ⁻¹ dry soil)	5.38 (1.59)	4.50 (0.51)	5.15 (0.84)	4.31 (1.00)	4.48 (0.30)	4.63 (0.58)	4.14 (0.35)
FUNG (nmol g ⁻¹ dry soil)	7.11 (1.49)	8.15 (1.37)	7.82 (1.35)	8.44 (3.04)	9.48 (2.38)	7.67 (0.36)	6.44 (0.35)
G + (nmol g ⁻¹ dry soil)	15.69 (3.50)	16.30 (2.25)	15.53 (2.89)	12.34 (5.45)	15.46 (1.98)	16.24 (0.40)	15.42 (0.81)
G - (nmol g ⁻¹ dry soil)	35.26 (16.35)	33.90 (2.15)	40.39 (9.24)	27.14 (15.54)	37.24 (7.10)	41.07 (15.54)	27.36 (4.10)
Saturated to unsaturated fatty acids	0.62 (0.09)	0.64 (0.06)	0.59 (0.04)	0.52 (0.16)	0.58 (0.12)	0.63 (0.00)	0.67 (0.06)

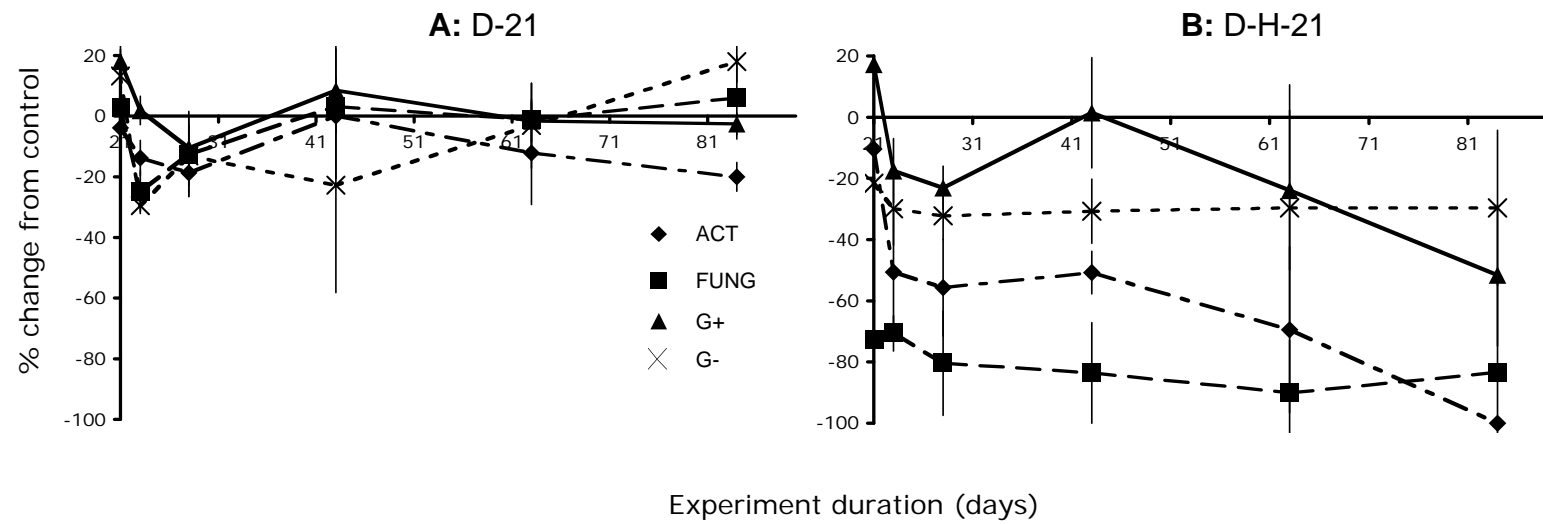
Table 2: Mean percentage change relative to control (standard deviation in brackets) for rate of soil respiration (n=4), SIR Glucose (n=4), concentration of total FAMES (n=3), ratio saturated to unsaturated fatty acids (n=3) and Bray and Curtis Indices (BCI) for CLPP (n=4) and FAMES (n=3), measured 2 days after the end of perturbation and 3 times perturbation duration after end of perturbation for Resistance and Resilience respectively.

Parameters	Measurements	D-2	D-H-2	D-7	D-H-7	D-21	D-H-21	D-H-21 repeated
Days of experiment	Resistance	4	4	9	9	23	23	23
	Resilience	9	9	28	28	84	84	84
Basal respiration	Resistance	-3.0 (12.9)	-3.8 (6.5)	0.9 (16.0)	14.1 (16.3)	124.0 (13.1)	140.8 (31.9)	51.6 (12.0)
	Resilience	17.7 (12.4)	16.7 (12.6)	36.7 (10.6)	-10.8 (16.3)	6.5 (4.1)	-14.4 (0.9)	-13.0 (3.3)
SIR Glucose	Resistance	-35.3 (3.6)	-31.4 (5.8)	-15.1 (6.4)	-11.8 (6.0)	-23.8 (2.9)	-31.3 (7.6)	-18.9 (6.5)
	Resilience	-8.9 (6.8)	-13.2 (9.7)	16.1 (5.7)	3.0 (5.0)	-3.5 (5.1)	-45.3 (2.2)	-34.03 (6.3)
CLPP-BCI	Resistance	0.903	0.908	0.863	0.917	0.850	0.883	0.937
	Resilience	0.918	0.873	0.910	0.864	0.955	0.854	0.843
Total FAMES	Resistance			-18.8 (9.5)	-8.8 (24.2)	-20.5 (3.7)	-34.2 (5.3)	
	Resilience			-0.2 (19.1)	-19.4 (27.4)	7.7 (6.3)	-47.9 (23.0)	
Saturated to unsaturated fatty acids	Resistance			-1.1 (17.5)	24.7 (15.4)	9.1 (1.9)	33.4 (6.9)	
	Resilience			2.5 (11.4)	6.9 (6.5)	1.3 (3.3)	-24.0 (35.7)	
FAMES-BCI	Resistance			0.855	0.836	0.828	0.736	
	Resilience			0.873	0.799	0.851	0.545	









6

