Soil Biology and Biochemistry 119 (2018) 59–62

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Short Communication

Drought in spring increases microbial carbon loss through respiration in a Mediterranean pine forest

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ARTICLE INFO

Keywords:
Decomposition
Climate change
Legacy effects of drought
Pinus brutia
Soil carbon

ABSTRACT

We investigated the effect of drought in spring (earlier onset of summer drought) on litter decomposition in a Mediterranean pine forest, in Greece. We exposed litterbags filled with decomposed or fresh pine litter in the forest floor from January to November and simulated drought from April to May (season with usually high activity in soil). The drought treatment resulted in a significant increase of microbial respiration and litter weight loss and a decrease of microbial C. Both litter types were similarly affected by drought, but differed in parameters related to decomposer community succession (Collembola and Acari abundances, organic C and N, microbial N). Our results indicate that drought during the highly biologically active season might have significant implications for soil Carbon sequestration/storage.

Decomposition is fundamental for ecosystem functioning and plays a key role in nutrient cycling (e.g. Hättenschwiler et al., 2005; Gessner et al., 2010). Climatic factors, litter quality and soil community composition and activity are the main factors influencing the decomposition process (Swift et al., 1979; Bradford et al., 2002, 2015; Handa et al., 2014). Longer and extremely dry periods are predicted to become more frequent in Europe, especially during the late spring and summer season (Christensen and Christensen, 2007; IPCC, 2007; Aiguo, 2013). These predictions also include the Mediterranean region where water is one of the limiting factors (Sardans and Peñuelas, 2007). We investigated the legacy effect of drought during spring (earlier onset of summer drought) on litter decomposition in a *Pinus brutia* Mediterranean pine forest. We hypothesized that drought during spring (i.e. highly biologically active season in Mediterranean systems) would affect litter decomposition patterns in the longer term (i.e. after several months), but that there might be differences among litters of different decomposition stage (corresponding to the different forest litter layers).

We carried our experiment out on Mt. Holomon (470 m a.s.l.) in N. Greece (see also Tsiafouli et al., 2005). The climate is Mediterranean with dry summers, cold and wet winters. Seasons of spring and autumn are relatively warm and wet (Fig. 1). We collected freshly fallen *P. brutia* needles from the study area by traps, 6 months before using them for the litterbags. Half of the material was air-dried (fresh) and the other half was aged (aged) in the laboratory. For aging, the litter was inoculated with a suspension containing fungi from the experimental area, incubated in darkness for 3.5 months at 20 °C, and regularly

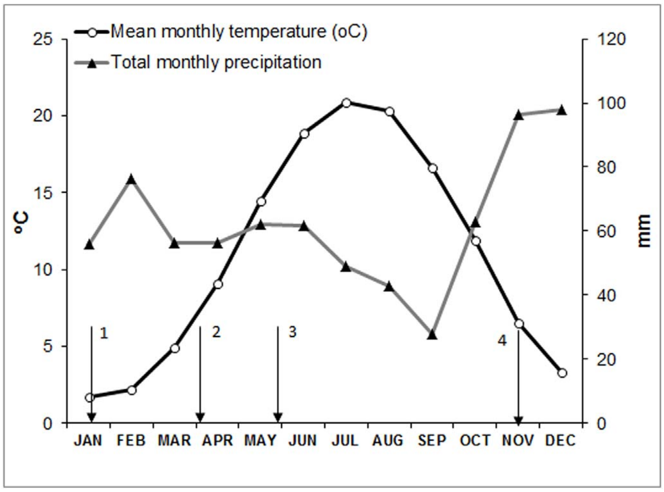

Fig. 1. Diagram of temperature and precipitation of the study area (Mt. Holomon, Greece) and experimental manipulation. The arrows depict stages of the experimental manipulation in the field: 1. Placing of litterbags on the forest layer, 2. Placing of roofs on top of the litterbags (drought plots), 3. Removal of roofs, and 4. Sampling of litterbags.

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https://doi.org/10.1016/j.soilbio.2018.01.010
Received 3 October 2017; Received in revised form 9 January 2018; Accepted 15 January 2018
Available online 20 January 2018
0038-0717/

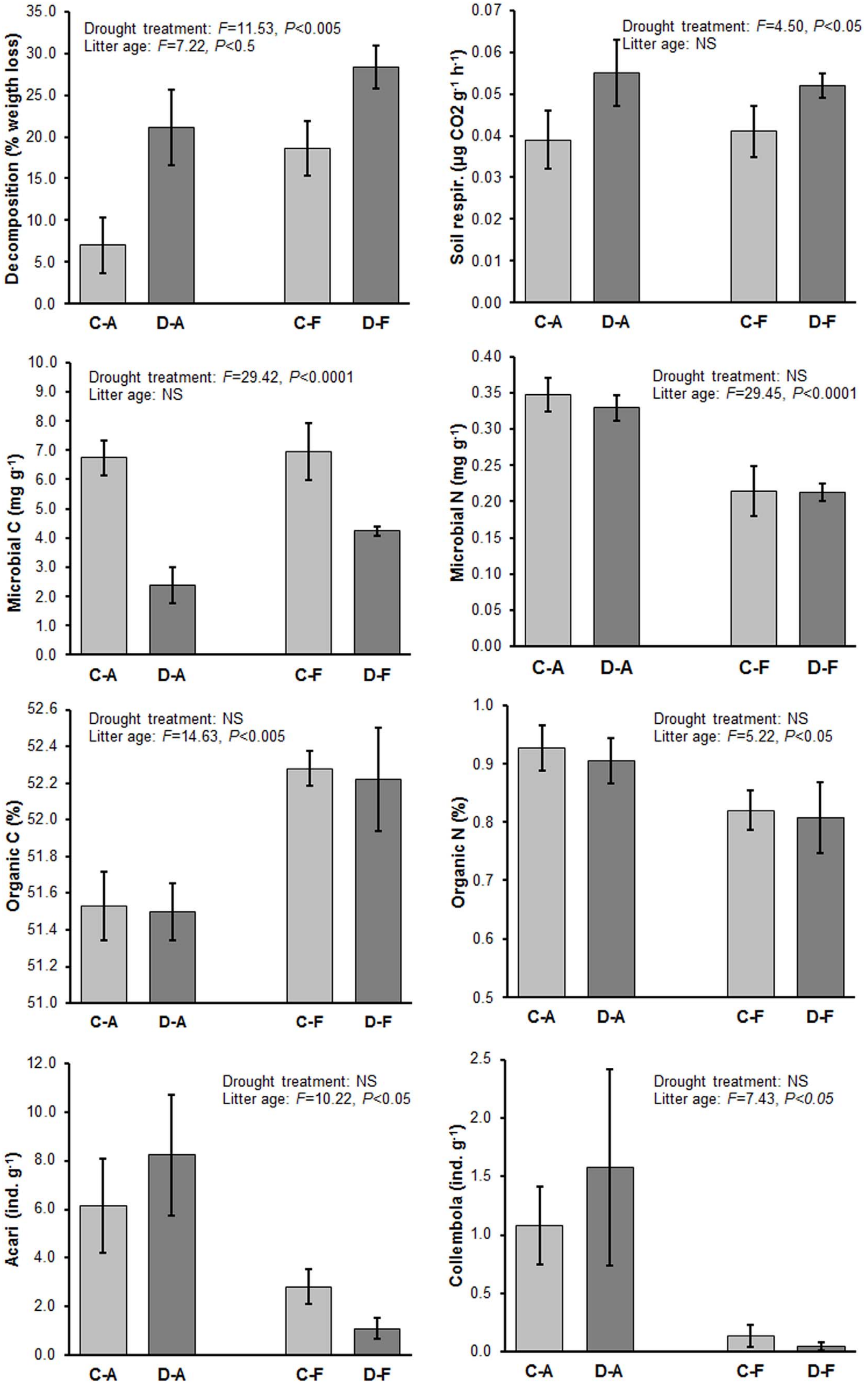

Fig. 2. Mean values of measured parameters, along with the effect of drought treatment and litter age as indicated by two-way ANOVA. The interactive effect of the independent factors was non-significant for all parameters, i.e. the effect of drought was similar for both litter types, and therefore is not shown in the graphs. Bars above columns indicate standard errors. Codes: the first letter and the color refer to the treatment (C, light grey: control; D, dark grey: drought). The second letter refers to the litter decomposition stage (A: aged, F: fresh). NS: non-significant.

moistened at > 60% water content. Litterbags with 1000 μm of mesh size (excluding macrofauna) were filled with fresh or aged litter, corresponding to 50 g dry weight. Litterbags were placed between the L and F-layer in January in six plots (2 replicates of each litter type per plot) randomly distributed in the study area.

In three of the six plots, we used roofs (125 cm × 175 cm size) to intercept natural precipitation (80 cm height with a small declination). Roofs were placed over the drought plots at the beginning of April and were removed at the end of May (the start of summer drought) (see Fig. 1). The three control plots (without roofs) received natural precipitation. We collected litterbags in November (same year) and we measured % litter weight loss, organic C and N (Allen, 1974), microbial respiration (Ohlinger et al., 1996), estimated microbial C (Jenkinson and Powlson, 1976 as modified by Ross, 1990) and microbial N (Brookes et al., 1985), as well abundances of Acari and Collembola (extracted from litter by a Berlese funnel). All measurements were made on the litter of the litterbags. Water content was measured at the end of the drought treatment (in litter surrounding litterbags within the plot, in May) and in each sample (in litter from the litterbag, in November). Our data were analyzed (SPSS v 22 software package) by two-way ANOVA under Glm and by Principal Component analysis (PCA).

The implemented experimental set-up was highly effective in lowering the moisture levels of the drought plots (17.37%) throughout the spring period (April to May) reaching almost 50% lower soil moisture than that of the control plots (35.4%). Mean values of measured parameters, along with the effect of litter age and drought treatment, as indicated by two-way ANOVA, are presented in Fig. 2, while the ordination of samples and parameters on the PCA biplot is depicted in Fig. 3.

Litter age significantly affected organic C and N, microbial N, litter weight loss, and Collembola and Acari abundances. The fresh litter had higher organic C values (higher C/N), whilst the aged litter higher organic N (lower C/N) and higher microbial N values resulting in lower microbial C/N (microbial C did not differ). In the PCA (Fig. 3) fresh litter samples are ordinated towards the left side of the biplot along with organic C, while the right side is occupied by samples from the aged litter plots along with microbial N, organic N, and Collembola and Acari abundances. Differences observed are related to differential decomposition of the various complex compounds in fresh (e.g. more labile carbohydrates) and aged litter (e.g. more stable lipids) (Taylor and Wolters, 2005; Creamer et al., 2015), which are accompanied by changes in the structure of the microbial communities (Papatheodorou et al., 2012). The advance of decomposition favored Collembola and Acari abundances, which is expected as microarthropods feed on decomposer microorganisms, and hence are related to later successional stage of the decomposer community (Bardgett et al., 2005).

As a result of the drought treatment several decomposition-related parameters (microbial respiration, microbial C and litter weight loss) were significantly affected and these effects were obvious several months after the ending of the treatment both in the fresh and the aged litter. Microbial respiration increased by 27% in the former and by 41% in the latter, leading to a decrease of microbial C by 39% and 65% respectively compared to the control plots. Litter weight loss increased 52% in the fresh, and 201% in the aged litter. In the PCA (Fig. 3) along the vertical axis samples from the drought plots occupy the upper part along with % weight loss and respiration, while control samples are ordinated towards the lower part of the plot, determined by Microbial C.

One explanation for our results could be that the drought treatment was implemented in spring, a season with high biological activity in the soil of Mediterranean ecosystems, because of elevated temperature but also adequate moisture conditions (Stamou and Sgardelis, 1989). The sudden decrease in moisture may have caused this highly active microbial community to go into dormant stages and become inactive as during the dry periods most of the energy of microbes is allocated to accumulate the solutes required to overcome osmotic stress (Schimel et al., 2007). The microbial community became highly active again when moisture conditions were favorable in autumn as rewetting causes a quick peak of microbial activity and growth (Fierer and Schimel, 2002).

On the other hand drought prolongation after late summer as applied in a previous study (Wilkinson et al., 2002) in the same study area had not such significant effects on decomposition related parameters. One explanation for this contrast is because soil communities in Mediterranean ecosystems are adapted to seasonal drought and contain historically selected stress-tolerant microbes, which may be though less abundant and less active throughout the year, but are the main microbes active during the summer drought period (Fierer et al., 2003;

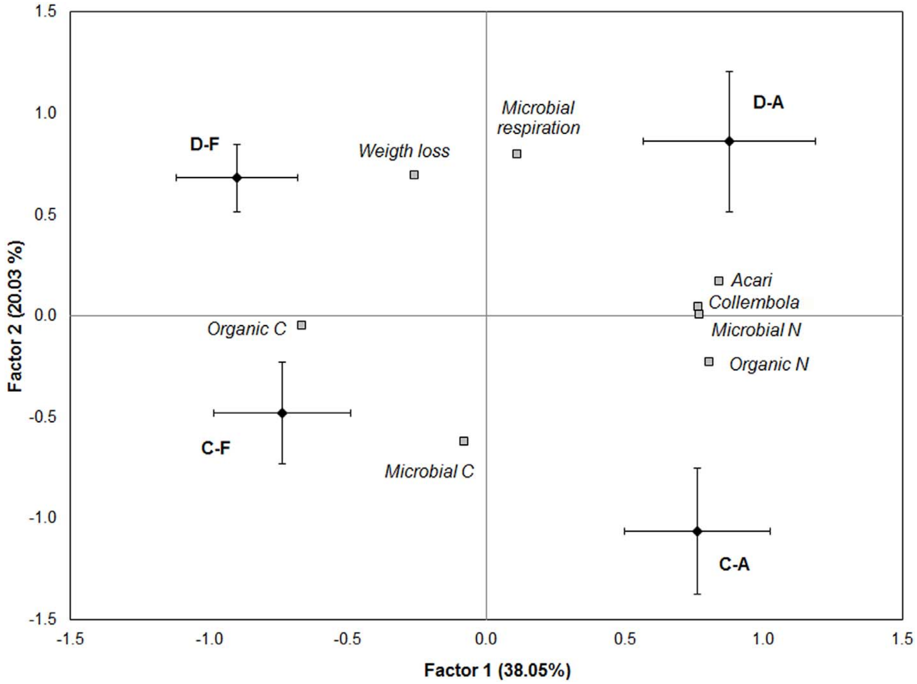

Fig. 3. PCA biplot showing ordination of samples (mean values indicated by dark symbols with st. er. bars) and parameters (indicated by bright symbols). The first two axes of the PCA account for 58.08% of the data variability (the variability explained by the first axis is 38.05%, while that explained by the second is 20.03%). Codes as in Fig. 2.

Fioretto et al., 2008). In the aforementioned study the microbial community went through a gradual decrease of moisture (before summer) and ended up with a different synthesis after summer drought. Hence the microbial energy status, which determines whether Carbon is employed in new microbial biomass or CO_2 release, is different compared to the present study where the "non-adapted" microbial community went through drastic moisture oscillations.

In conclusion, our study showed that drought during the season of the year with high biological activity has legacy effects even after several months. It leads to the increase of litter weight loss and loss of microbial carbon through the increase in respiration, regardless of the stage of litter decomposition. Hence future climate change related to earlier onset of summer drought in the Mediterranean might have significant implications for soil Carbon sequestration and storage.

Acknowledgements

Equipment used was funded by the former EU project GLOBIS, (ENV4-CT95-0027).

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