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# Responses of carbon, nitrogen and phosphorus to two consecutive drying–rewetting cycles in soils

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## Abstract

Drying and rewetting cycles are known to be important for the dynamics of carbon (C), phosphorus (P), and nitrogen (N) in soils. This study reports the short-term responses of these nutrients to consecutive drying and rewetting cycles and how varying soil moisture content affects microbial biomass C and P (MBC and MBP), as well as associated carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) emissions. The soil was incubated for 14 d during which two successive drying–rewetting episodes were imposed on the soils. Soils subjected to drying (DRW) were rewetted on the seventh day of each drying period to return them to 60% water holding capacity, whilst continually moist samples (M), with soil maintained at 60% water holding capacity, were used as control samples. During the first seven days, the DRW samples showed significant increases in extractable ammonium, total oxidized nitrogen, and bicarbonate extractable P concentrations. Rewetting after the first drying event produced significant increases only in CO<sub>2</sub> flux (55.4 μg C g<sup>-1</sup> d<sup>-1</sup>). The MBC and MBP concentrations fluctuated throughout the incubation in both treatments and only the second drying–rewetting event resulted in a significantly MBC decrease (416.2 and 366.8 mg kg<sup>-1</sup> in M and DRW soils, respectively).

The two drying–rewetting events impacted the microbial biomass, but distinguishing the different impacts of microbial *versus* physical impacts of the perturbation is difficult. However, this study, having a combined approach (C, N, and P), indicates the importance of understanding how soils will react to changing patterns of drying–rewetting under future climate change.

**Key words:** drying–rewetting / emissions / nitrous oxide / microbial biomass / soil nutrients

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## 1 Introduction

Soils are exposed to natural drying and rewetting cycles which result in the perturbation of nutrient cycling, mainly resulting from changes in microbial activity (De Nobili et al., 2006; Butterly et al., 2010) and diversity within the microbial community (Fierer et al., 2003; Steenwerth et al., 2005; Gordon et al., 2008; Butterly et al., 2009). Understanding the effects of drying and rewetting events is therefore important so that the impact of changing rainfall patterns and increased temperature (anticipated due to climate change) on soil nutrient cycles, particularly in agricultural systems, can be predicted. Previous studies have shown that the rewetting of dry soil stimulates microbial activity, resulting in increased mineralization of soil organic matter (SOM) (Fierer and Schimel, 2003) and pulses of carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) emissions along with enhanced macronutrient availability (Franzluebbers et al., 2000; Wu and Brookes, 2005; Butterly et al., 2010, 2011). Fierer and Schimel (2003) observed that drying and rewetting cycles affected soil carbon (C) cycling in the short term by increasing the microbial mineralization of cytoplasmic solutes accumulated during drying, with a spike in soil respiration after rewetting when microbial

communities re-grew in response to available nutrients. During rewetting, those microorganisms that passively equilibrated to the dry conditions are able to rehydrate (Kieft et al., 1987) and are then able to multiply rapidly exploiting the increase in available nutrients (Nguyen and Marschner, 2005). Butterly et al. (2009) reported that a single drying and rewetting cycle may also result in a reduction in microbial biomass carbon (MBC) and phosphorus (MBP). In addition, Gordon et al. (2008) observed that drying and rewetting stress significantly reduced the quantities of MBC, fungal phospholipid fatty acids (PLFAs) and microbial biomass nitrogen in both low and high productivity grassland soils.

Where drying and rewetting cycles are regular events (becoming part of a long-term trend), it has been shown that indigenous microbial communities may change in response to changes in soil moisture potential (Griffiths et al., 2003; Steenwerth et al., 2005; Butterly et al., 2009). Fierer and Schimel (2003) observed that drying and rewetting processes affect soil C cycling in the longer-term through exposure of SOM in micro-aggregates, which open-up upon drying.

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Drying has also been related to changes in soil structure, causing aggregate destruction and increased respiration upon subsequent rewetting events (Navarro-García et al., 2012). Previously it had been thought these micro-aggregates might become resistant after experiencing successive drying and rewetting cycles, resulting in some physical protection of SOM from microbial utilization (Denef et al., 2001a). However, it was demonstrated that frequent episodes of soil drying and rewetting can affect both C and N transformations in ways that increase the likelihood of losses of these elements from the soil to air and water (Fierer and Schimel, 2002; Mikha et al., 2005), causing reductions in SOM, soil microbiota, and soil fertility.

It is known that soil drying and rewetting can lead to increased losses of nutrients via leachates. Gordon et al. (2008) found that drying and rewetting increased concentrations of dissolved organic C and dissolved organic N in leachates collected from grassland soils, which received regular applications of fertilizers. Similarly, Fierer and Schimel (2002) reported a decrease in N availability with time following soil drying and rewetting due to stimulation of microbial growth and attendant N immobilization. Blackwell et al. (2013), investigating the effect of the rate of rewetting dried soils on nutrient concentrations in leachate, reported a little effect of 0- and 4-h treatments on the total oxidized N concentrations in leachates from dried soils, whereas in the 24-h rewetting rate resulted in a significant decrease. Moreover, Blackwell et al. (2013) reported a decrease in the concentrations of P compounds in leachates from dried soil with a more rapid rewetting rate, possibly as a result of increased microbial activity over time and changing soil physical properties. Moreover, Nguyen and Marschner (2005) found that drying and rewetting events resulted in a rapid increase in both MBP and labile organic P, and these effects were exacerbated in soils with high organic matter content.

Based on the evidence cited above, it is clear that identifying the impacts of changing patterns of drying and rewetting episodes due to climate change on nutrient cycling within soils is crucial to our understanding of how soil fertility will change. In this study, we examined the influence of two consecutive drying and rewetting cycles on soil C, N and P, and MBC and MBP dynamics. Few research projects have looked at this range of parameters in soil drying–rewetting studies, usually focusing on just one or two of the macro-nutrients or either dissolved or gaseous exports, but not in combination as we have done here. The aim of this study was to increase our understanding of how repeated drying–rewetting cycles affect nutrient availability and cycling in the short term (14 d). It was hypothesized that drying and rewetting affect the cycling of C, N, and P in a similar way, and that their availability is linked to the response of the microbial biomass. We also investigated the effect of drying–rewetting cycles on greenhouse gases (CO<sub>2</sub> and N<sub>2</sub>O) in order to study the relationships between nutrient cycling and microbial activity under these conditions.

## 2 Material and methods

### 2.1 Soil properties and collection

The soil used in the experiment was collected from Rothamsted Research's North Wyke site in Devon, South-west UK. Characterized as a clayey, non-calcareous typichaplaquept (USDA) of the Hallsworth Series (FAO dystic Gleysol), this soil was described in detail by Harrod and Hogan (2008).

Approximately 4 kg of soil were collected using a 10-cm diameter soil corer. Soil cores were collected randomly from a permanent grassland field to a depth of 10 cm. Immediately after sampling, the soil was prepared as described by Blackwell et al. (2013), Sun et al. (2007), Iovieno and Bååth (2008), who tested the variations of nutrients, microbial community composition, and bacterial growth, respectively, in dried and rewetted soils. Briefly, in our experiment the soil was passed through a 2-mm sieve and after removing all visible root material and stones, it was stored at 4°C for 1 week prior to further chemical analysis and use in incubations. The main physico-chemical characteristics of this soil are reported in Tab. 1.

### 2.2 Incubation experiment

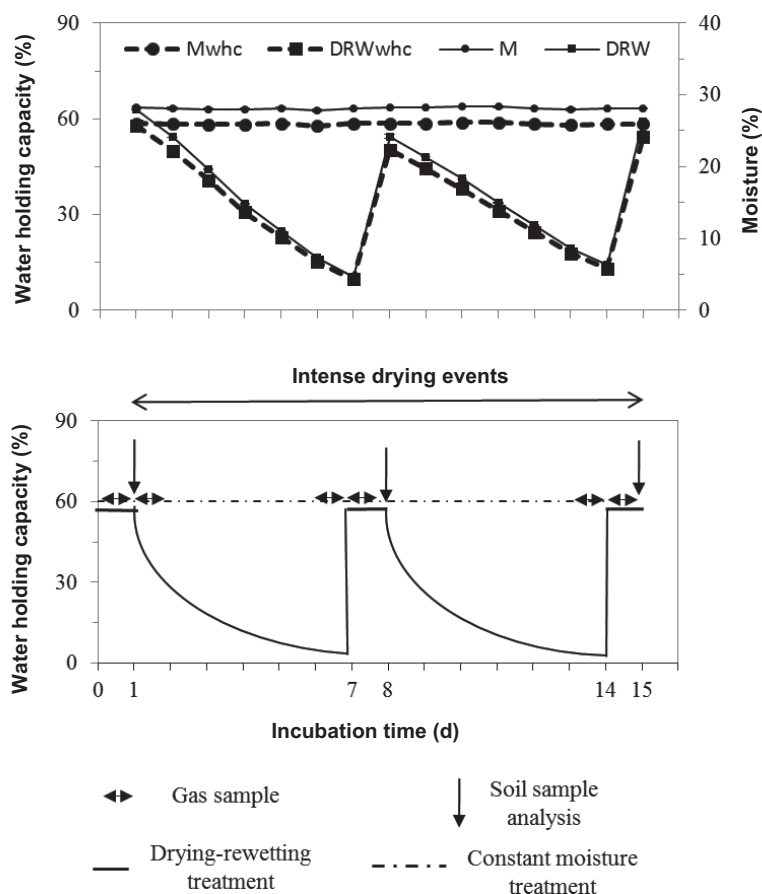
The experimental design consisted of a factorial arrangement with two moisture regimes (M and DRW for constant moisture and drying–rewetting treatments, respectively) and three sampling times (Fig. 1).

Water holding capacity (WHC) was measured as described by Dane and Hopmans (2002) and soil moisture was adjusted to a standardized water content (60% WHC) using ultrapure deionized water (hereafter referred to as deionized water) to

**Table 1:** Initial soil properties (0–0.10 m) of the Hallsworth soil (mean ± SE, *n* = 3).

Parameters	
Water Holding Capacity (%)	57.1
Moisture (%)	27.5 ± 0.0
Total organic C (%)	2.8 ± 0.0
Water extractable MRP (mg P kg <sup>-1</sup> )	0.50 ± 0.03
Water extractable TP (mg P kg <sup>-1</sup> )	2.7 ± 0.5
Bicarbonate extractable P (mg P kg <sup>-1</sup> )	3.4 ± 0.1
KCl extractable TON (mg N kg <sup>-1</sup> )	n.d. <sup>a</sup>
KCl extractable NH <sub>4</sub> <sup>+</sup> (mg N kg <sup>-1</sup> )	n.d.
MBP (mg P kg <sup>-1</sup> )	263.9 ± 28.6
MBC (mg C kg <sup>-1</sup> )	798.2 ± 31.0
pH	5.9

<sup>a</sup>n.d., no detectable



**Figure 1:** Soil moisture content during the drying–rewetting regimes and sampling time. Error bars show mean  $\pm$  SE ( $n = 3$ ).

activate microbial biomass without suturing the soil. Equal amounts (100 g dry weight equivalent) of soil were added to 0.5-L Kilner jars and soil moisture was re-adjusted to 60% WHC. Soils were pre-incubated for 8 d in the dark at 20°C, during which moisture was maintained at 60% WHC, and perforated lids were used to limit moisture evaporation. Subsequently, the lids were removed and two successive drying–rewetting episodes were imposed on the DRW soils, drying lasting 7 d at 20°C in an incubator. On the seventh day of each drying period, deionized water was added to all samples to return them to 60% WHC before randomized destructive sampling was carried out to provide samples for analyses. M soil samples, with soil maintained at 60% WHC by weighing and addition of appropriate quantities of deionized water each day, were used as reference samples for all determinations. Three randomly selected replicate samples were destructively used at this same point 24 h after wetting and analyzed for KCl extractable  $\text{NH}_4^+$  and total oxidized N (TON), water extractable molybdate reactive (MRP), and total P (TP), bicarbonate extractable P, MBC and MBP, extractable organic C (EOC), and soil moisture content.

### 2.3 Laboratory analyses

The KCl extractable total oxidized N (TON), which includes nitrate ( $\text{NO}_3^-$ ), and nitrite ( $\text{NO}_2^-$ ), and  $\text{NH}_4^+$  concentrations were measured by extracting 14 g moist weight of soil in

28 mL of 2 M KCl solution on an orbital shaker for 1 h and filtering through Whatman no. 2 filter papers (Whatman plc., Maidstone, UK) using the method of *Bremner and Keeney* (1966). Concentrations of TON and  $\text{NH}_4^+$  in extracts were measured using a discrete photometric analyser (Thermo-Fisher Aquakem 250, Loughborough, UK), following the methods described by *Kempers and Luft* (1988) and *Searle* (1984), respectively.

For measurement of water extractable molybdate reactive P (MRP) and total P (TP), 14 g of fresh soil were extracted in 40 mL of ultrapure water (Milli RQ Water Systems, Millipore, Bedford, Ma, USA) and shaken at 150 rpm on an orbital shaker for 1 h, followed by centrifugation at 16.9 g ( $r_{\text{max}}$  15.13 cm) for 10 min and filtered using Whatman no. 42 filter paper. For determination of TP, water extracts were digested (*Rowland and Haygarth*, 1997) prior to analysis for MRP and the orthophosphate concentrations were determined in samples according to the method by *Murphy and Riley* (1962) using a discrete photometric analyzer (Thermo-Fisher Aquakem 250, Loughborough, UK).

Bicarbonate extractable P was measured following the method developed by *Olsen et al.* (1954), during the determination of MBP using the  $\text{CHCl}_3$  fumigation method described by *Brookes et al.* (1982). Briefly, non-fumigated and fumigated samples (including P-spiked samples to account for soil adsorption of released P) were extracted in 0.5 M  $\text{NaOHCO}_3$  (adjusted to pH 8.5) in the ratio of 1 : 20 (w/v) dry weight equivalent soil to extract.

MBC was measured according to the methodology by *Gregorich et al.* (2000), extracting chloroform fumigated and non-fumigated samples in ultrapure water, using a  $K_{\text{ec}}$  value of 0.35 (*Sparling et al.*, 1990). Extractable organic C (EOC) was obtained from the non-fumigated control samples.

Soil pH was measured in 0.05 M  $\text{CaCl}_2$  with a pre-calibrated Thermo Orion pH meter (Model 420) at a 1 : 2 soil : solution ratio.

### 2.4 $\text{CO}_2$ and $\text{N}_2\text{O}$ emissions measurements

For measurement of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  fluxes, the Kilner jar lids were fitted with two rubber septa to avoid leaks and so that gas samples could be taken from headspace. After the 8-d pre-incubation (initial samples) and on day 7 and 14 of the DRW assay, 3 replicates (Kilner jars) were used for the determination of  $\text{CO}_2$  and  $\text{N}_2\text{O}$  emissions after sealing the jars over a 60-min period, with samples being collected at 0, 30, and 60 min. This was carried out before and after the soil samples had been rewetted, with the first  $t = 0$  minute gas sample taken immediately following the soils rehydration to 60% WHC. Gas samples were injected using a syringe into pre-evacuated 22-mL Perkin Elmer crimped capped headspace vials, fitted with chlorobutyl rubber seals. These headspace samples were then analyzed using a Perkin Elmer Clarus 500 Gas Chromatograph linked to a TurboMatrix 110

headspace autosampler. The instrument was optimized for detection of  $N_2O$  and  $CO_2$  using two Elite-Q PLOT megabore capillary columns (30 m  $\times$  0.53 mm) in parallel and two detectors: an Electron Capture Detector (ECD) for determination of  $N_2O$  and a Flame Ionization Detector (FID), fitted with a methanizer, to detect  $CO_2$ . Calibration and instrument performance standards used were obtained from the British Oxygen Company (BOC), and Air Products.

## 2.5 Statistical analysis

Significant between treatment means values were calculated in ANOVA using Genstat (*VSN International*, 2015). Tukey's HSD test was used to describe grouping of the treatment means as significant at the  $p \leq 0.05$  level. A correlation among all the parameters was evaluated by a two-sided test of correlation.

Canonical variate analysis (CVA) was used to find the set of variables that best distinguish among multiple groups of parameters. From CVA analysis between treatments multivariate distances were used to form a similarity matrix and using this matrix a cluster analysis dendrogram was constructed (using Average Linkage Criterion).

All data are expressed on a soil dry weight basis and the results are reported as the mean of three replicates  $\pm$  standard error (SE).

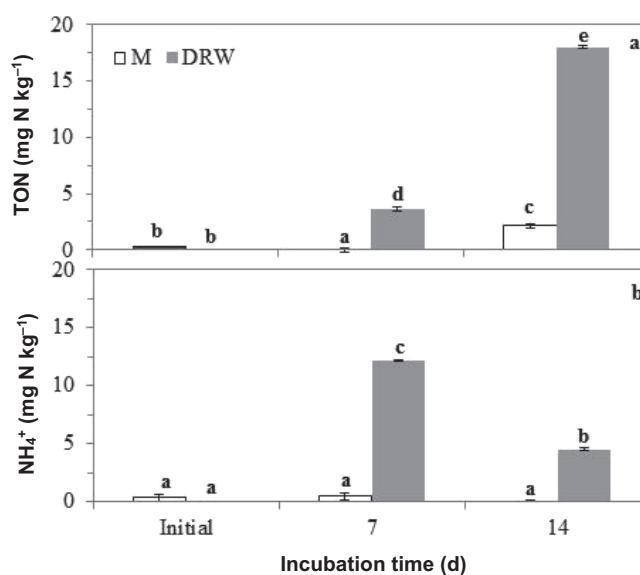
## 3 Results

### 3.1 Initial soil properties

The soil properties prior to the commencement of the first drying and rewetting episode were analyzed for all initial samples. Both KCl extractable TON and KCl extractable  $NH_4^+$  were present but only in trace amounts in all initial soils. Water extractable MRP and TP concentrations were relatively low, but MBP (114.5  $\pm$  7.1 and 118.5  $\pm$  3.8 mg P kg<sup>-1</sup>, respectively, in M and DRW initial soils) and MBC (650.8  $\pm$  17.3 and 633.2  $\pm$  32.5 mg C kg<sup>-1</sup>, respectively, in M and DRW initial soils) were similar in both sets of samples. Bicarbonate extractable P concentrations were low (4.3  $\pm$  0.1 and 3.7  $\pm$  0.1 mg kg<sup>-1</sup>, respectively, in M and DRW initial soils) equating to an Olsen P soil index of 0 (based on UK RB209 Fertiliser Recommendations; *Defra*, 2010).

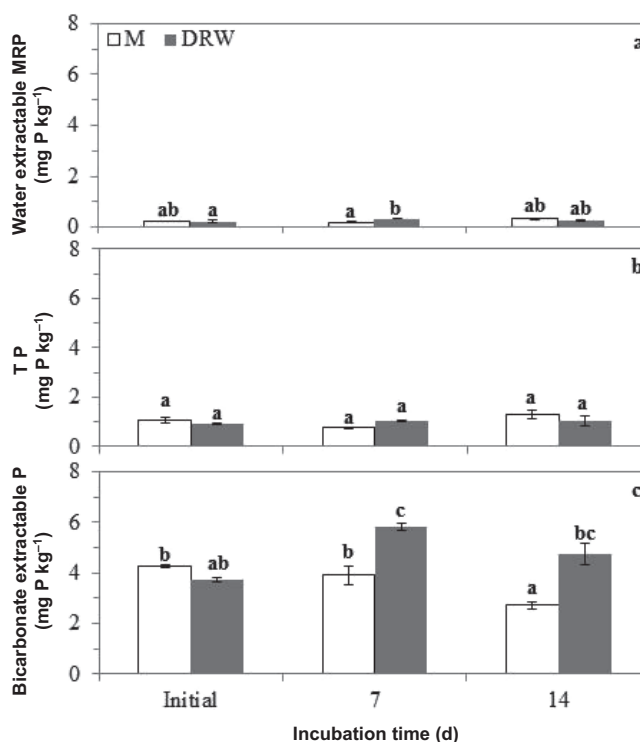
### 3.2 Effect of soil drying on N, P, and C

The effect of soil drying–rewetting on TON and  $NH_4^+$  concentrations is reported in Fig. 2a, b. Initial samples had very low concentrations of both TON and  $NH_4^+$  (both < 1 mg N kg<sup>-1</sup>). After the first drying episode (days 1–7), the significant reduction ( $\approx$  80%) in soil moisture was accompanied to the significant increases ( $p \leq 0.05$ ) in concentrations of both compounds (3.6  $\pm$  0.1 and 12.2  $\pm$  0.1 mg N kg<sup>-1</sup> for TON and  $NH_4^+$ , respectively) in the DRW samples. The second drying episode (day 8–14) caused a reduction in soil moisture of  $\approx$  75% and a further increase in TON concentrations to 18.0  $\pm$  0.3 mg N kg<sup>-1</sup>, whilst  $NH_4^+$  concentrations decreased to 4.5  $\pm$  0.5 mg N kg<sup>-1</sup>.

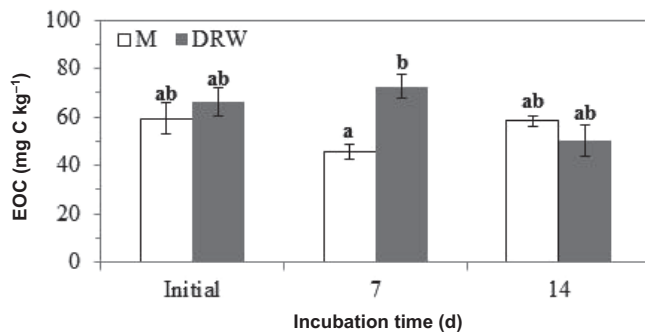


**Figure 2:** TON (a) and  $NH_4^+$  (b) concentrations in control soil samples (M) and dried then re-wetted soils (DRW) during the incubation period. Error bars show mean  $\pm$  SE ( $n = 3$ ). Superscript letters indicate significant differences as derived from Tukey HSD test ( $p \leq 0.05$ ) following ANOVA.

Concentrations of water extractable MRP and TP (Fig. 3a, b) varied very little in both the M and DRW treatments, despite the imposition of the drying–rewetting episodes, with a significant difference ( $p \leq 0.05$ ) between the M and DRW treat-



**Figure 3:** Water extractable MRP (a), TP (b), and bicarbonate extractable P (c) concentrations in control soil samples (M) and dried then re-wetted soils (DRW) during the incubation period. Error bars show mean  $\pm$  SE ( $n = 3$ ). Superscript letters indicate significant differences as derived from Tukey HSD test ( $p \leq 0.05$ ) following ANOVA.



**Figure 4:** Extractable Organic C concentrations in control soil samples (M) and dried then re-wetted soils (DRW) during the incubation period. Error bars show mean  $\pm$  SE ( $n = 3$ ). Superscript letters indicate significant differences as derived from Tukey HSD test ( $p \leq 0.05$ ) following ANOVA.

ments only after 7 d in MRP. In contrast, bicarbonate extractable P concentration (Fig. 3c) increased in the DRW samples compared to the M treatment following the period of drying between day 1 and day 7, increasing from an initial concentration of  $3.7 \pm 0.1$  to  $5.8 \pm 0.1$   $\text{mg kg}^{-1}$ , whereas the bicarbonate extractable P concentrations in both M and DRW treatments decreased following the second drying episode ( $2.7 \pm 0.1$  and  $4.8 \pm 0.4$   $\text{mg kg}^{-1}$  for M and DRW, respectively).

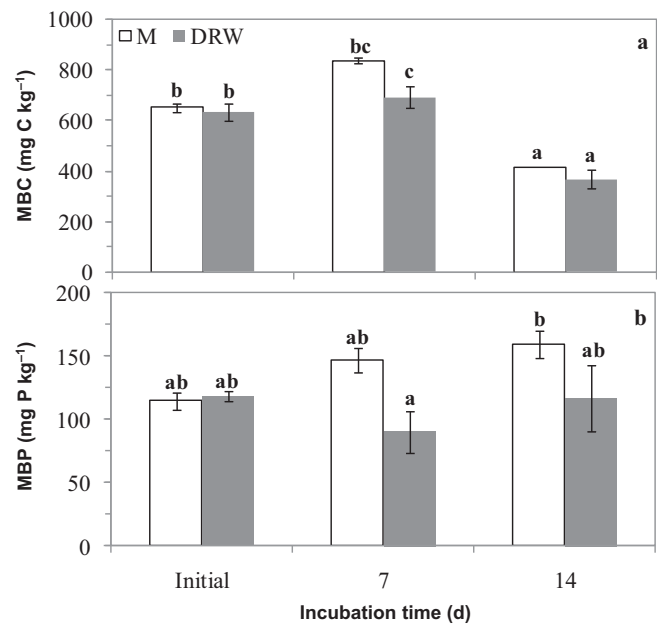
After the first period of drying, EOC concentration (Fig. 4) showed a small increase only in the DRW treatment, but it was not significant. Afterwards, no such difference occurred in the second drying episode.

### 3.3 Effect of drying on microbial biomass C and P

During the first period of drying a small increase in MBC in both M and DRW treatments occurred that was significant ( $p \leq 0.05$ ) in DRW treatment ( $837.6 \pm 12.0$  and  $693.0 \pm 44.1$   $\text{mg kg}^{-1}$  for M and DRW, respectively) (Fig. 5a). The second period of drying resulted in a significant decrease ( $p \leq 0.05$ ) in concentrations to approximately half those measured after the first drying episode. Although there was considerable fluctuation in concentrations in both the M and DRW samples throughout the experiment, the MBC in the M samples was always higher than in the DRW samples even if no significant differences were observed between the two treatments. The MBP concentrations (Fig. 5b) in the M samples increased gradually throughout the incubation from an initial value of  $114.5 \pm 7.1$  to  $159.3 \pm 10.7$   $\text{mg kg}^{-1}$  after the second drying event. The MBP in the DRW samples declined after the first drying event to  $90.2 \pm 16.7$   $\text{mg kg}^{-1}$  and then recovered slightly to  $117.1 \pm 26.1$   $\text{mg kg}^{-1}$  following the second event.

### 3.4 Effect of drying and rewetting cycles on CO<sub>2</sub> and N<sub>2</sub>O emissions

The CO<sub>2</sub> and N<sub>2</sub>O emissions were evaluated at the beginning of the experiment and on day 7 and 14 in both M and DRW samples before and after rewetting. There was little variation in the fluxes of CO<sub>2</sub> from both M and DRW treatments



**Figure 5:** Microbial biomass C (a) and P (b) in control soil samples (M) and dried then re-wetted soils (DRW) during the incubation period. Error bars show mean  $\pm$  SE ( $n = 3$ ). Superscript letters indicate significant differences as derived from Tukey HSD test ( $p \leq 0.05$ ) following ANOVA.

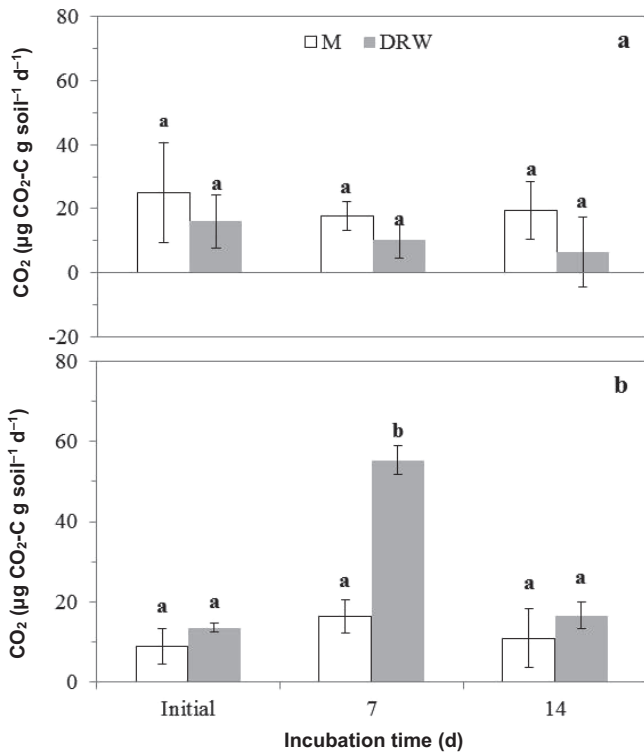
throughout the incubation, including both before and after rewetting, with fluxes ranging from 6.5 to 55.4  $\mu\text{g CO}_2\text{-C g soil}^{-1} \text{d}^{-1}$  (Fig. 6a, b). The CO<sub>2</sub> flux before rewetting showed a gradual but not significant decrease compared to the initial soils. In contrast, the DRW treatment showed a significant increase in CO<sub>2</sub> flux ( $p \leq 0.05$ ) after rewetting on day 7, increasing from  $13.6 \pm 1.0$   $\mu\text{g CO}_2\text{-C g soil}^{-1} \text{d}^{-1}$  before rewetting to  $55.4 \pm 3.7$   $\mu\text{g CO}_2\text{-C g soil}^{-1} \text{d}^{-1}$  after rewetting.

There were no significant differences in N<sub>2</sub>O fluxes between M and DRW treatments before rewetting and overall standard errors were large (Fig. 7a). However, there was a trend to decrease in flux in both treatments, particularly in DRW treatment after rewetting (Fig. 7b) and this was most pronounced after rewetting on day 14 ( $-10.4 \pm 2.5$   $\text{ng N}_2\text{O-N g soil}^{-1} \text{d}^{-1}$ ), but this difference was not significant.

## 4 Discussion

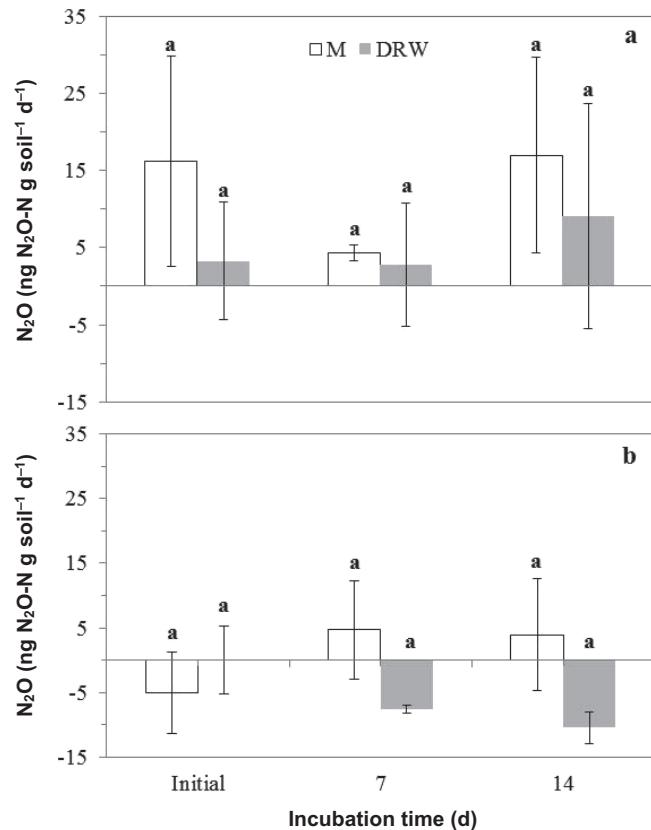
### 4.1 Effect of soil drying/wetting regimes on N dynamics

The two drying and rewetting cycles had different effects on nutrient availability during the 14 d of incubation. More specifically, the reduction in moisture in the DRW treatment resulted in an increase in mineral N, both of NH<sub>4</sub><sup>+</sup> and TON forms. The increase in NH<sub>4</sub><sup>+</sup> after 7 might be due to the mineralization of SOM or also to the release of NH<sub>4</sub><sup>+</sup> from the lysis of microbial cells after rewetting (e.g., Blackwell et al., 2013). Likely, the increase of NH<sub>4</sub><sup>+</sup> was due to the disruption of soil aggregates and loss of physical protection of SOM (Adu and Oades, 1978). Although prior to start the experiment the soil was sieved at 2 mm, Thomson et al. (2010) suggested that sieving



**Figure 6:** CO<sub>2</sub> emissions before (a) and after rewetting (b) in both control soil samples (M) and dried then re-wetted soils (DRW). Error bars show mean ± SE ( $n = 3$ ). Superscript letters indicate significant differences as derived from Tukey HSD test ( $p \leq 0.05$ ) following ANOVA.

fresh soil to this size is not sufficient to physical disruption and to the release of organic matter. Whereas drying and rewetting events may enhance the aggregate turnover, especially in the first drying-wetting cycles (Deneff et al., 2001a) and may cause the exposition of SOM to microbial attack, supplying pulses of substrates to microorganisms (Xiang et al., 2008). The mineralization of the SOM since the first drying episode had little effect on MBC concentrations compared to those in the initial sample. The relative decrease in MBC following the second drying episode may explain the TON increase seen at that time, but this may not be due to the drying event itself, because a similar trend was observed in the M samples. Whilst the increase of TON in the second drying period probably was due to the oxidizing conditions of soil, which affect the transformation of  $\text{NH}_4^+$  in  $\text{NO}_3^-$  form. The increased availability of organic substrates derived from microorganisms or SOM might have stimulated N mineralization (Miller et al., 2005). In addition, Gordon et al. (2008) showed that the increase in dissolved inorganic and organic N concentrations in leachates occurred especially in soils which received regular applications of fertilizer. In our study, an increase in  $\text{NH}_4^+$  concentration occurred after the first period of intense drying only. Afterwards, the aerobic conditions in the DRW treatment would have promoted nitrification of  $\text{NH}_4^+$ , causing the subsequent increase of TON concentration at the end of the incubation. This is shown by the increase in TON following the second drying period and a concomitant decrease in  $\text{NH}_4^+$ . This suggests that more than half of the  $\text{NH}_4^+$  was oxidized, contributing significantly to the increase of



**Figure 7:** N<sub>2</sub>O emissions before (a) and after rewetting (b) in both control soil samples (M) and dried then re-wetted soils (DRW). Error bars show mean ± SE ( $n = 3$ ). Superscript letters indicate significant differences as derived from Tukey HSD test ( $p \leq 0.05$ ) following ANOVA.

TON in the soil. Indeed, Fierer and Schimel (2002) found a significant increase in autotrophic nitrifiers in stressed oak woodland and grassland soils, probably because of their ability to survive periods of low moisture. This suggests that the enhanced rates of nitrification may result in increased leaching losses of TON and gaseous losses of N via nitrification or denitrification. However, it was demonstrated that extractable  $\text{NO}_3^-$  concentration decreased when soils were exposed to multiple drying-rewetting cycles (15 stress events), as well as net N mineralization in a Norway spruce forest soil (Fierer and Schimel, 2002; Muhr et al., 2010). Borken and Matzner (2009), in a review study on the mechanisms that induce changes in N mineralization by drying and rewetting, reported that the fate of ammonium and nitrate is not well known and the large variations of results may be explained by differences in experimental design or also can change under field conditions. Overall, it can be stated that N availability for plant depends by on duration and the intensity of drying-rewetting events and their effect on microbial activity. He and Dijkstra (2014) demonstrated that drought stress affected more N limitation on plant growth in the short-term (< 90 d), probably due to the reduced microbial activity and N mineralization that occur with the reduction of soil moisture.

#### 4.2 Effect on soil P and C

The drying–rewetting cycles affected bicarbonate extractable P in a similar way to  $\text{NH}_4^+$  concentrations, while both water extractable MRP and TP were relatively unaffected by the soil drying. Turner and Haygarth (2001) showed an increase of water soluble P after drying and rapidly rewetting, and most of it was in organic forms. This phenomenon was probably due to the P released from the lysed cells of microorganisms (Salema et al., 1982). Moreover, Turner and Haygarth (2003) showed that bicarbonate extractable P increased after drying, suggesting that for bicarbonate extractable P, changes in P solubility might be more important than direct release from the microbial biomass. In fact, the P pulse might be of non-biomass origin, likely due to the solubilization of non-living organic matter and release of adsorbed inorganic P (Butterly et al., 2009). Therefore, in our experiment, drying may have had an important effect on soil structure and, hence, aggregate disruption, resulting in new substrates becoming available. Borken and Matzner (2009) stated that this was probably due to the presence of soil macroaggregates that are destabilized during drying and this effect on C mineralization is limited to few dry–wet cycles. In addition, Denef et al. (2001a) reported that soils subjected to drying–rewetting cycles initially had enhanced aggregate turnover, but after several cycles the aggregates became stable. Therefore, given that there was little change in MBC and MBP in our experiment after the first drying–rewetting event, it is possible that previously protected SOM became exposed to microbial attack following aggregate breakdown, as demonstrated by Denef et al. (2001b). These findings support the hypothesis the increase of EOC in DRW treatment after the first drying–rewetting event may be due to the release of physically protected organic matter. Afterwards EOC reduction during the second drying event may be attributable to its mineralization. Xiang et al. (2008) suggested that drying and rewetting regimes have a range of effects on soil C dynamics, leading to a cascade of responses, such as soluble C release and biomass growth, resulting in metabolization of unavailable soil C, particularly in subsurface soils. In addition, Beare et al. (2009) observed an increase in dissolved organic C after rewetting of uncompacted and compacted soil led to increased  $\text{CO}_2$ -C production, suggesting a link between dissolved organic matter availability and C mineralization following rewetting of dried soils. The results suggested that few drying–rewetting events might induce to C loss caused by the increase of soil respiration rate for the enhanced microbial activity. However, the changes of available organic matter content could not be considered the only explanation of the long-term respiration decrease in frequently stressed soils (Fierer and Schimel, 2002).

#### 4.3 Microbial biomass C and P

Soil drying and rewetting cycles can influence microbial activity and biomass, and the effect depends on the number of DRW cycles (Zhao et al., 2010). Generally, dry soils after wetting induce the hydration and lysis of dead microbial cells which accumulate during drying periods (Borken and Matzner, 2009). In our experiment, the MBC decreased after the second drying event in both treatments, suggesting that fac-

tors in addition to the DRW events were driving MBC dynamics. This indicates that the drying–rewetting events might have a greater impact only after several events, probably due to certain resilience of microorganisms to disturbances (Griffiths and Philippot, 2013). It is well known that land use and the addition of exogenous organic matter can cause changes in microbial biomass C and affect the microbial community structure (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008; Jangid et al., 2008; Pezzolla et al., 2013). Fierer and Schimel (2002) demonstrated that drying–rewetting events did not alter the size of microbial pool immediately after the stress treatment, but can induce to an increase of microbial biomass C after several drying–rewetting events, resulting in the long-term biomass accumulation. Zhang et al. (2007) attributed the increase of soil microbial biomass to a shift of microbial community from bacterial dominance to fungal dominance with the frequency and intensity of wetting and drying within 60 d of incubation. There are contrasting results concerning the effect or repeated drying–rewetting events on MBC: microbes may decrease due to DRW stress or increase for the increased substrates released during DRW process for the breakdown of occluded soil organic C in the aggregates (Van Gestel et al., 1993; Denef et al., 2001a). In addition, Sawada et al. (2017) suggested that DRW stress histories of soils may be essential to model microbial controls on soil C dynamics. The variation of microbial community structure in our experiment can only be partially explained by MBC results, however, the use of biomarkers or molecular approach might be more appropriate for studying the community composition (Insam, 2001; Pezzolla et al., 2015).

The small decrease of MBP in the DRW treatment after the first drying event is further evidence of the impact of DRW on the microbial biomass. In our study, correlation between MBP and MRP was not significant ( $r = -0.20$ ;  $p = 0.48$ ), not supporting the hypothesis that P was released from lysed cells. Butterly et al. (2009) observed a similar trend, with a gradual depletion of microbial biomass C and P after consecutive drying events. The MBC and MBP concentrations followed different trends, with the MBC being relatively unaffected by the first event, but declining after the second although not necessarily due to soil drying (see above), whilst the MBP clearly decreased after the first event, but recovered slightly following the second event. The resulted C : P ratio of soil microbial biomass is lower with respect to the common values found in literature (15–60; Brookes et al., 1984; Cleveland and Liptzin, 2007), however, the results reflected almost the values found by Blackwell et al. (2013) in the Hallsworth soil. Although as best estimate of C : P ratio of soil microbial biomass are 42.4 at global scale, it was also true that soil microbial biomass can vary in a wide range among biomes and there are a number of uncertainties in its measuring (Xu et al., 2013). In our study, the different dynamics of MBC and MBP implied the variations of C : P ratio, ranging from 2.6 to 8.1, reaching the minimum values at the end of the experiment for both M and DRW samples, which was likely due to changes in microbial community composition. Drying–rewetting may reduce bacterial growth and gram-negative bacteria seem to represent the main microbial source of the P pulse, while fungal growth remained unaffected (Bapiri et al., 2010; Dinh et al., 2017). Heuck et al. (2015) reported that the microbial biomass C : N : P



ratios may be altered by changing environmental conditions, especially in P-poor soils, probably due to shifts in microbial community composition.

It is not possible to know if the microbial biomass recovered following the first drying event or if the biomass vulnerable to drying–rewetting was killed and a stable, resilient population remained, thus, meaning the second drying event had much less impact. However, the fact that elevated bicarbonate P concentrations were measured in the DRW treatments relative to the M treatments following both drying–rewetting events does suggest that further microbial release of P did occur. This can have important implication on soil P dynamics, since microorganisms C-limited could mineralize organic P, leaving inorganic P in the soil and increasing the availability to plants (Heuck et al., 2015).

#### 4.4 CO<sub>2</sub> and N<sub>2</sub>O emissions

Rewetting a dry soil typically causes a pulse of respiration, a phenomenon known as the “Birch Effect” (Birch, 1958), especially when intense drying has occurred. Soil respiration rates can be affected by the nature of the drying and rewetting regimes, which can cause different soil aeration rates and changes in water filled pore space. The variation of water content can affect the soil C mineralization rates both in the short (up to 1 week) and long term (up to 6 weeks) (Fierer and Schimel, 2002). The only significant change in CO<sub>2</sub> emissions observed during this experiment was immediately after the first rewetting event. This was also observed by Beare et al. (2009), who showed an increase in CO<sub>2</sub> flux from soil immediately following rewetting, especially in uncompacted soil, and this flux corresponded with an increase in EOC. Moreover, Fierer and Schimel (2002) showed that the frequency of drying and rewetting events can affect CO<sub>2</sub> emissions, demonstrating that the exposure to these frequent events decreased the amount of CO<sub>2</sub> released upon rewetting, probably because of a decrease in the supply of remaining mineralizable organic matter, again as in our study. The peak of CO<sub>2</sub> flux in the DRW treatment following rewetting after the first drying event is perhaps the most dramatic result in this experiment. A five-fold increase in CO<sub>2</sub> flux occurred compared to that prior to rewetting in DRW, although the different moisture content observed at the end of the first drying episode between the two treatments. This increase corresponded to an increase in soil NH<sub>4</sub><sup>+</sup> concentration, suggesting mineralization of the organic matter occurred concurrent with microbial growth when soil moisture decreased. However, the second drying–rewetting event did not cause a similar increase in emissions, which could be due to stabilization of the microbial activity resulting in nitrification of the NH<sub>4</sub><sup>+</sup> and resulting in an increase in NO<sub>3</sub><sup>-</sup>. This suggests that consecutive drying–rewetting events do not result in the same effects, while the initial drying significantly affects microbial population dynamics, the subsequent drying periods result in less drastic changes due to stabilization or adaptation of the microbial population.

This trend partially agrees with Zhao et al. (2010), who found that basal soil respiration was gradually reduced by drying–rewetting cycles, based on the assumption that a portion of

microorganisms could survive the drying stress. These microorganisms probably decrease their resilience to drying after several drying–rewetting events, affecting not only CO<sub>2</sub> emissions but also the capacity of N<sub>2</sub>O reduction to N<sub>2</sub>. Our results supported the hypothesis that generally the microbial community can survive two drying and rewetting cycles, as showed by the lack of significant differences between parameters in the DRW and M treatments at the end of the experiment, but it did not test if some microorganisms, such as denitrifiers, were affected as previously found for an arable soil that was subject to drought (Bergstermann et al., 2011). In addition, the negative correlation between CO<sub>2</sub> emissions after rewetting and MBP was  $r = -0.55$  ( $p \leq 0.05$ ) suggests that the drying and rewetting cycles may have an influence on microbial activity, as probably explained by the small decrease of MBP after the first drying event.

The N<sub>2</sub>O emissions observed during the experiment were positively correlated to MBC after the rewetting events ( $r = 0.52$ ,  $p \leq 0.05$ ), indicating that the low emissions were likely due to the decrease in microbial activity. Even Muhr et al. (2008) showed very low N<sub>2</sub>O emissions (close to zero) in dried forest soils, probably attributable to the less activity of nitrifying and denitrifying microorganisms. It is also well known that soil water content is one of the dominant variables controlling N<sub>2</sub>O emissions together with N supply. Indeed, Skiba and Smith (2000) observed that rainfall affects water filled pore space and, hence, the N<sub>2</sub>O fluxes from soil, since a high degree of anaerobicity is required to produce N<sub>2</sub>O emissions via denitrification. This might explain the higher (although not significantly so) fluxes from the M treatment compared to the DRW treatment before rewetting, suggesting that a constant amount of water content can positively affect N<sub>2</sub>O emissions. Moreover, Mikha et al. (2005) showed that repeated drying and rewetting cycles may lead to a reduction of mineralized C and N due to a reduction in microbial activity. Although N<sub>2</sub>O emissions after rewetting were negatively correlated to TON ( $r = -0.50$ ,  $p = 0.06$ ), it can be hypothesized that the N<sub>2</sub>O fluxes were probably related more closely to other factors affecting denitrification rates and not the availability of TON. Bergstermann et al. (2011) found that dry soils, when rewetted, produced larger N<sub>2</sub>O emissions compared to soils that were kept wet. This did not apply in our study, in which no significant differences occurred between DRW and M soils, both before and after rewetting. The N<sub>2</sub>O fluxes results suggest that two consecutive drying–rewetting cycles did not affect the dynamics of microbial activity. Only the enhanced nitrification process occurred with the second drying–rewetting cycle might have influenced the increased N losses as NO<sub>3</sub><sup>-</sup> or as gaseous N (Fierer and Schimel, 2002).

#### 4.5 Effect of drying and rewetting cycles on soil properties

In the perspective of climate changes scenarios and the possible effect on nutrients dynamics in the Mediterranean area, it was important to consider simultaneously all parameters studied in this experiment, in order to examine the response of nutrients after two consecutive drying–rewetting cycles.

Figure 8 shows how the samples were distributed based on the results obtained from all the analyses. Although two consecutive drying–rewetting events can be considered as a short period to test the microbial dynamics, as previously stated for  $N_2O$ , it is important to note that all DRW treatments showed different characteristics respect to M samples, especially for TON, bicarbonate extractable P,  $NH_4^+$ , and  $CO_2$  after rewetting. This is particularly true for  $NH_4^+$  and TON, confirming that the drying and rewetting cycles had an important effect on mineral N dynamics in the short-term period. Zhao et al. (2010) found that soil  $NO_3^-$ -N concentration did not differ with moisture regime cycle and its decrease was probably due to the decrease in microbial biomass following the DRW cycles. This was not observed in our experiment, in which the MBC and MBP behavior were only partially affected by the two drying–rewetting events. Sawada et al. (2017) suggested that the reductions of microbial C biomass may depend on the different tolerance of soil microorganisms. Regarding the emissions, only  $CO_2$  fluxes after the second rewetting showed a significant response to the moisture regime at 7 d, suggesting that the soil moisture drove the microbial activity only after the first drying–rewetting event, probably due to the temporary C supply to microbes that enhanced the pulse of respiration (Xiang et al., 2008). This evidence can explain the small increase of EOC values in DRW samples after the first drying–wetting event. This effect was negligible at the end of the experiment, and Evans and Wallenstein (2011) suggested this might be due to the historical soil moisture that plays a role in the microbial responses. The lack of difference in  $N_2O$  fluxes in the two treatments did not allow considering this parameter suitable to explain the N dynamics after two consecutive drying–rewetting cycles. Concerning the parameters to evaluate P variations, only bicarbonate extractable P was affected by the moisture regimes. Overall, the PCA analyses shows that the determination of mineral N forms, EOC, and  $CO_2$  fluxes parameters might be useful to explain the short-term variations of nutrients availability after two consecutive drying–rewetting cycles.

The dendrogram (Fig. 9) shows an overview of the shifts in properties in the treatments and at different times. The fact that M-7 (representing the parameters for the M treatment after 7 d) has about 50% similarity to DRW-7 (representing the DRW treatment after 7 d) shows that the first drying event had an important effect on the two treatments, but also that there had been a shift in the value of parameters from those in the initial sample (a natural drift) and that this shift was greater than the effect of the drying. It also illustrates that the second drying event (DRW-14) caused a major shift in soil parameters, as overall parameters were again quite different. Therefore,

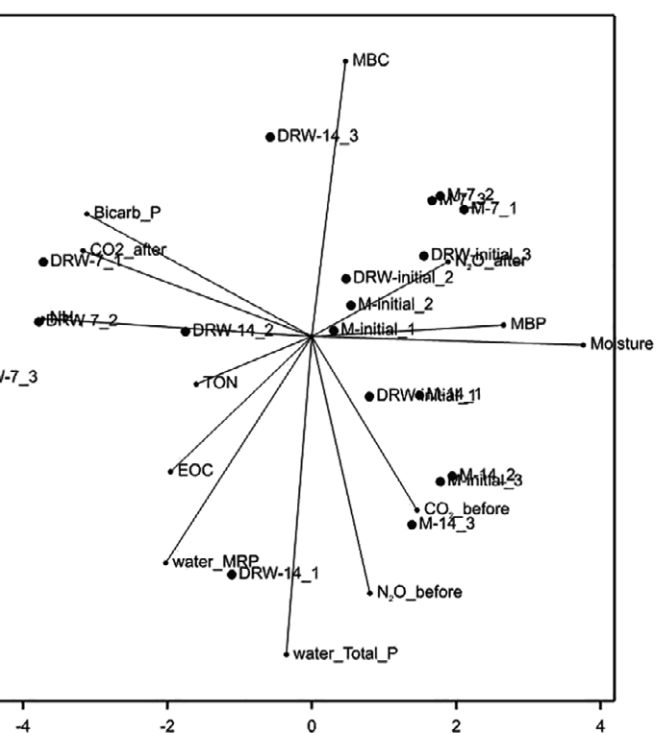


Figure 8: Principal component biplot of multiple groups of parameters and the distribution of all samples analysed [x-axis = principal component 1 (22.29%); y-axis = principal component 2 (17.18%)].

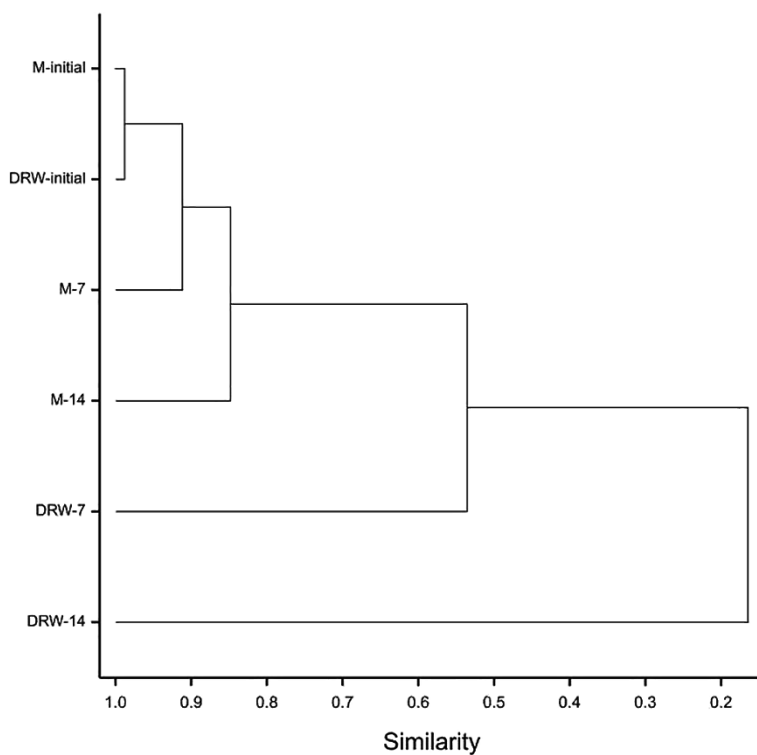


Figure 9: Dendrogram based on similarity matrix.

the similarity matrix demonstrates the effect of consecutive drying–rewetting events was more evident after the second drying event. However, the nature and direction of the chang-

es in soil properties are not necessarily the same following both events

## 5 Conclusions

This study has shown that the changes in soil properties resulting from two consecutive drying–rewetting events are complex and that the impact of soil drying can vary from one event to another, as the soil adapts to the changes that occur. Whilst some soil properties show evidence of having an initial resilience to soil drying (e.g., MBC), others appear to develop resilience following an initial impact from drying (e.g., MBP), whilst other properties appear to demonstrate cumulative impacts. However, the impact of two consecutive drying–rewetting events is cumulative, depending on many factors including microbial dynamics. The complexity of the results in this study may be due in part to the fact the drying events weren't extreme and further work is required to determine the soil moisture thresholds at which particular impacts occur (e.g., cell lysis), and this is likely to vary greatly among different soils.

An increased awareness of how soil fertility and plant nutrients availability might be influenced by more frequent drying rewetting cycles is the first step in understanding how the resilience of soil needs managing in order to mitigate such shifts in soil functionality.

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