# Repeated drying and rewetting differently affect abiotic and biotic soil phosphorus (P) dynamics in a sandy soil: A ^{33}P soil incubation study

Hao Chen [a,1], Klaus A. Jarosch [b,1,*], Éva Mészáros [c], Emmanuel Frossard [c], Xiaorong Zhao [a,**], Astrid Oberson [c]

[a] College of Land Science and Technology, China Agricultural University, Key Laboratory of Plant-Soil Interactions, Ministry of Education, Key Laboratory of Arable Land Conservation (North China), Ministry of Agriculture, Beijing 100193, China
[b] Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012, Bern, Switzerland
[c] Institute of Agricultural Sciences, ETH Zurich, Eschikon 33, CH-8315, Lindau, Switzerland

## ARTICLE INFO

## ABSTRACT

Soil drying and rewetting (DRW) events are expected to occur at higher frequencies because of alterations in climate patterns. Readily extractable inorganic and microbial soil phosphorus (P) pools may be affected due to rapid changes in soil water availability. We aimed to determine how soil P dynamics are affected by repeated soil DRW using a sandy grassland soil that regularly experiences DRW. In a laboratory soil incubation study, the soil was exposed to three DRW cycles, with each cycle consisting of a two-day drying phase, a three-day dryness phase and a four-day moist phase after rapid rewetting. The indicators of abiotic processes (P sorption) and biotic processes (respiration, microbial abundance, potential phosphatase enzyme activities) were regularly determined together with water-extractable P, resin-extractable P and microbial P in a ^{33}P-labelled soil.

During the first DRW cycle, microbial P was reduced by half and accompanied by a concomitant but not equivalent increase in water-extractable P and a slight as well as delayed increase in resin-extractable P. Thus, increases in water-extractable P were explained by microbial P released during drying but also by microbial P occupying soil P sorption sites, thereby decreasing soil P sorption. Changes in the ^{33}P-isotopic composition of microbial P at the same time suggested that microorganisms did not respond homogenously to the DRW treatment and indicated an increased mineralisation of previously unavailable organic P compounds. However, during the second and third DRW cycles, only water-extractable P, soil P sorption and potential phosphatase activities were affected by the DRW treatment, whereas all other parameters remained similar in values to the constant moist treatment. The effects of DRW on soil P dynamics appeared to affect water-extractable P more long-lastingly, whereas microbial P and most of the biotic indicators quickly adjusted to the DRW treatment. We conclude that the current concepts suggesting an increased mobility of soil P towards other environmental compartments due to soil DRW should consider that abiotic and biotic soil P dynamics are not equally affected in the case of short repetition of DRW incidences.

## 1. Introduction

Drying and rewetting (DRW) of soil is a common natural perturbation that affects the dynamics of nutrients, such as phosphorus (P), in soil (Blackwell et al., 2010). Repeated DRW incidences are predicted to become more frequent due to global climatic change (IPCC, 2014), and alterations in soil DRW periods are expected to affect soil P dynamics and the mobility of P within soil (Forber et al., 2018).

Soil P dynamics are governed by abiotic and biotic processes. Abiotic processes include the sorption and desorption of P from the soil solid phase and the precipitation and dissolution of P-bearing minerals (Frossard et al., 2000). Biotic processes include the hydrolysis of organic P compounds by phosphatase enzymes and the microbial immobilisation of inorganic or mineralised organic P (Richardson and Simpson, 2011).

---

* Corresponding author. Institute of Geography, Hallerstrasse 12, CH-3012, Bern, Switzerland.
** Corresponding author. Key Laboratory of Arable Land Conservation (North China), Ministry of Agriculture, Key Laboratory of Plant-Soil Interactions, Ministry of Education, College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, PR China.
*E-mail addresses:* klaus.jarosch@giub.unibe.ch (K.A. Jarosch), zhaoxr@cau.edu.cn (X. Zhao).
[1] These authors contributed equally to this work.

Although microbial biomass represents a relatively small soil P pool, which is usually 0.5%–7.5% of the total P in grassland soils (Oberson and Joner, 2005), microbial P fluxes have been shown to largely dominate gross P fluxes in soils with low inorganic P availability (Bünemann et al., 2012; Schneider et al., 2017). The availability of P for microbial and plant uptake is usually estimated with the amount of water-extractable P or anion exchange resin absorbable P in soil-water suspensions (Yli-Halla et al., 2016).

The DRW of soil has a large effect on soil P dynamics, as both abiotic and biotic processes can be affected. Increases in the range of doubling the water-extractable P have been commonly observed as a result of DRW in incubation studies using homogenised soil (Blackwell et al., 2010) and undisturbed soil, where soil aggregation is preserved (Hömberg and Matzner, 2018). This increase is usually due to the physical disruption of soil aggregates, which render available the P that was previously protected and not extractable (Bünemann et al., 2013). However, the disaggregation of soil upon DRW can also increase the number of P sorption sites in soil, as reported by the increase in the P sorption capacity of soils after soil DRW (Olsen and Court, 1982; Haynes and Swift, 1985a, b). However, an increase in soil P sorption capacity can decrease the water-extractable P levels, suggesting that not only abiotic but also biotic processes exert a large influence on water-extractable P during DRW.

Increases in water-extractable P are strongly related to the release of P from lysed microbial cells induced by DRW (Blackwell et al., 2010; Achat et al., 2012b). For example, Turner and Haygarth (2001) found a positive correlation between the increase in water-extractable P during DRW and the amount of microbial P in different grassland soils. Another biotic process affecting water-extractable P is the hydrolysis of organic P compounds catalysed by phosphatase enzymes. However, both increases and decreases in the potential activities of phosphatases during soil DRW were reported (Speir and Ross, 1981; Zornoza et al., 2006). Moreover, the increased availability of carbon after DRW (Butterly et al., 2009) could promote the microbial immobilisation of P from soil solution, potentially decreasing water-extractable P values. Distinct soil microbial communities seem to differ in susceptibility to being negatively affected by DRW (Dinh et al., 2017; Preece et al., 2019). Thus, a characterisation of both, changes in P pool sizes (e.g. microbial P, water-extractable P) and the related fluxes between these pools is necessary to understand the underlying processes in how DRW affects soil P dynamics.

Given the large effect of microbial P on soil P dynamics and water-extractable P, the determination of microbial P turnover rates (i.e. the renewal of P bound in the microbial biomass) is essential to understand the microbially mediated P dynamics during DRW. Microbial P turnover can be derived from net changes in the size of the pool by dividing the sum of losses over a given period of time by the average microbial biomass (McGill et al., 1986). This concept has been widely applied in field investigations. Microbial turnover rates of 0.8–1.3 year$^{-1}$ were found in New Zealand and European grassland soils (Chen et al., 2003; Liebisch et al., 2014). Conversely, Chen et al. (2018) found faster turnover rates of 3.0–3.6 year$^{-1}$ in a semiarid grassland in north China with more drastic fluctuations in soil moisture than in other studies. However, fluctuations in the size of microbial P do not capture the concurrent growth and death of soil microbes with little net changes in microbial P and may thus underestimate microbial P turnover (Oberson and Joner, 2005).

To determine soil P fluxes, isotopic pool dilution can be a useful tool. Studies using P radioisotope techniques have found important P fluxes through the soil microbial biomass in the absence of net changes in the size of microbial P. For example, a few days after labelling the isotopically exchangeable P in a grassland soil under conditions of constant soil moisture and temperature, 25%–46% of the P radioisotope was recovered in a constant microbial P pool, suggesting a dynamic equilibrium between P uptake and release from the soil microbial biomass (Oberson et al., 2001; Bünemann et al., 2012). Yevdokimov et al. (2016) found an intensive microbial immobilisation (up to 41%) of a $^{33}$P spike added after rewetting air-dried soils. Using a $^{32}$P tracer, Chepkwony et al. (2001) found a decreased specific activity (SA, i.e. $^{32}$P/$^{31}$P or $^{33}$P/$^{31}$P) in soil resin-extractable P (an indicator of available P) by subjecting soil to DRW cycles and interpreted this as an increase in organic P mineralisation due to DRW. However, a thorough understanding of microbial P dynamics and their link to water-extractable P under repeated DRW cycles (including phases of drying, dryness and rewetting) remains lacking.

Thus, we conducted an incubation study to improve the understanding of how repeated DRW affects the pools and processes governing soil P dynamics. To this end, we selected a sandy soil under semiarid grassland that commonly experiences DRW as a model system. A $^{33}$P soil labelling experiment was conducted in combination with monitoring soil P pools (water-extractable P, resin-extractable P and microbial P). Abiotic (soil P sorption) and biotic (microbial P turnover, soil respiration, bacterial and fungal abundance, phosphatase activities) indicators affecting soil P dynamics were also determined.

## 2. Material and methods

### 2.1. Site description and soil preparation

Soil samples were taken in August 2016 from a long-term grassland grazing enclosure experiment in Inner Mongolia, China (N 43°38′, E 116°42′). The sampling site is located in a semiarid zone (Chen et al., 2018), with an annual mean precipitation of 280–350 mm and annual evaporation of 4–5 times that of the precipitation (315 mm). The uneven precipitation distribution, especially in the rainy season (summer), subjects the topsoil to DRW regularly. The soil is a Calcic-Orthic Aridisol (Calcic Chernozem according to ISSS Working Group RB 1998), with a sandy texture composed by more than 80% sand. *Stipa grandis* (P.A. Smirn.) and *Leymus chinensis* (Trin.) are the typical vegetation species in this region (Chen et al., 2018).

For the incubation experiment, we selected a sub-plot (20 m × 150 m) in an enclosure treatment, excluded from grazing since 1983. After removing the litter layer, 15 randomly distributed soil cores (0–20 cm depth) were taken and homogenised to obtain a composite sample. In the laboratory, the soil was air dried, and coarse fresh plant debris was removed by sieving at 4 mm before incubation. Soil characteristics were determined in air-dried soil (Table 1) using milled soil for the analysis of total organic carbon, total nitrogen, total P and organic P to minimise analytical variation.

Prior to incubation, the air-dried soil was pre-incubated in the dark at 20 °C for 17 days, with the gravimetric water content maintained at 0.23 g g$^{-1}$ (equivalent to 45% of the water holding capacity [WHC] and to a water potential of about pF 2.1). Pre-incubation was conducted to reach basal respiration conditions (expressed in mg $CO_2$ kg$^{-1}$ d$^{-1}$). During this period, soil respiration was measured every two days to confirm that the microbial activity was constant by the onset of incubation.

### 2.2. Soil incubation

After pre-incubation, a 30-day incubation experiment was started. The incubation involved two treatments: 1) constant moist (CM), in which the soil moisture was kept constant at 0.25 g g$^{-1}$ (equivalent to 50% WHC and pF 2), and 2) DRW (three cycles in total, nine days for each cycle), which started on day 3 (a three-day equilibrium phase after soil labelling and mixing). Each cycle included a two-day drying phase (decrease in soil water content from 0.25 to 0.05 g g$^{-1}$, equivalent to a change in water potential from pF 2 to 4.2), a three-day dryness phase (pF 4.2) and a rapid rewetting followed by a four-day wet phase (from pF 4.2 to 2 and then maintained at pF 2) (Fig. 1). Incubation was conducted in a climate chamber at 20 °C in the dark. All treatments were set up with three replicates and in a fully randomised design.

In detail, on day 0, pre-incubated moist soil (equivalent to 2050 g dry

**Table 1**

Characteristics of the semiarid grassland soil (0–20 cm sampling depth) used in the study. Means ± standard errors of three replicates are presented.

| Parameter | Unit | Value |
|---|---|---|
| Sand[a] | mass % | 82.2 ± 1.0 |
| Silt[a] | mass % | 11.1 ± 0.6 |
| Clay[a] | mass % | 6.7 ± 0.6 |
| pH[b] | | 7.3 ± 0.01 |
| WHC[c] | g $H_2O$ g$^{-1}$ soil | 50.6 ± 0.4 |
| Bulk density[d] | g cm$^{-3}$ | 1.20 ± 0.02 |
| Total organic C[e] | g C kg$^{-1}$ soil | 15.9 ± 0.5 |
| Total N[f] | g N kg$^{-1}$ soil | 1.44 ± 0.01 |
| Total P[g] | mg P kg$^{-1}$ soil | 298.7 ± 6.2 |
| Organic P[h] | mg P kg$^{-1}$ soil | 132.4 ± 1.9 |
| Olsen-P[i] | mg P kg$^{-1}$ soil | 2.6 ± 0.04 |

[a] Particle size distribution was measured by the pipette method (Sheldrick and Wang, 1993).

[b] pH was measured as a soil-water saturated paste (Miller et al., 1997).

[c] The maximum water holding capacity (WHC) was determined gravimetrically after gravitational water loss from the sample saturated with water.

[d] Bulk density was determined by oven drying the cutting ring of moist soils to a constant mass at 105 °C.

[e] Total organic C was measured by dichromate digestion (Kalembasa and Jenkinson, 1973).

[f] Total N was measured by Kjeldahl digestion (Bremner, 1965).

[g] Total P was determined by wet digestion using $H_2SO_4$ and $H_2O_2$ (Anderson and Ingram, 1993).

[h] Organic P is given as NaOH-EDTA extractable organic P (Cade-Menun and Preston, 1996).

[i] Olsen-P represents NaHCO$_3$ extractable P (Olsen et al., 1954).

soil) received 11 ml of a carrier-free $^{33}P$ labelling solution (as diluted phosphoric acid; Hartmann Analytics GmbH, Braunschweig, Germany) and additional $H_2O$ to increase the water content to 0.25 g g$^{-1}$ (pF 2), followed by manual stirring for 10 min using a plastic spoon and bowl. The amount of $^{31}P$ added with the $^{33}P$ tracer was negligible, and the resulting radioactive label (R) was equal to 9.36 kBq g$^{-1}$ soil. Aliquots of 40 g of moist, labelled soil were placed in each of 66 polyethylene containers (without lids, 3.5 cm in diameter and 4 cm in height), among which 24 (8 time points with 3 replicates) were used for the CM treatment and 42 (14 time points with 3 replicates) for the DRW treatment. For the CM treatment, one container with $^{33}P$-labelled soil and a beaker containing 10 ml of $H_2O$ (to maintain moisture during the incubation) were placed in a 2 L jar, which was then tightly closed, and the soil water content was maintained continuously at 0.25 g water g$^{-1}$ soil (monitored gravimetrically). For the DRW treatment, the bottom and wall of the container were evenly pierced to have 80 holes, with each hole

having a 2 mm diameter, and packed with filter paper (Whatman™, GE Healthcare, UK) prior to filling with soil to avoid soil loss through the holes. The pierced container was placed in a beaker (6 cm in diameter) containing 40 g of self-indicating silica gel (BDH Chemicals) surrounding the container. The gel was replaced once a day during the drying process. The suitability of this drying procedure was determined by a pre-test, in which the water content of the test soil was decreased linearly in a two-day drying process from pF 2 to 4.2. The soils were rewetted by simulating a short and intensive rain spell by gravimetrically adding $H_2O$ onto the soil using a pipette, allowing 1 min for each container. Soil moisture in each sampling during incubation was as expected, demonstrating that the DRW procedure worked well (Fig. S1).

In parallel, unlabelled soil was incubated under the same conditions, except for the use of an equivalent volume of $H_2O$ instead of a $^{33}P$ labelling solution to maintain the same initial soil moisture. Unlabelled soil was used to quantify soil respiration, whereas labelled soil was used to determine all of the other parameters measured throughout the incubation period (see section 2.3). For all measurements during the incubation (except for soil respiration), the soils were destructively sampled at each sampling time (Fig. 1). As a result, a total of 96 jars (30 jars with unlabelled soils and 66 jars with labelled soils) were obtained for the entire incubation experiment.

### 2.3. Analytical methods used for measurements during the incubation

#### 2.3.1. Water-extractable P

The methods used for determining $^{31}P$ and $^{33}P$ concentration in water-extractable P and microbial P pools are given in Bünemann et al. (2016). Briefly, water-extractable P was determined by shaking moist soil equivalent to 10 g dry soil in 100 ml $H_2O$ (accounting for the water contained in the soil) for 16 h on an overhead shaker. The suspension was passed through a 0.2 μm cellulose acetate membrane filter (Minisart, Sartorius). The concentration of phosphate ions in the filtered solution (water-extractable $^{31}P$) was determined colourimetrically at 610 nm using the malachite green method, which determines the molybdate-reactive P (Ohno and Zibilske, 1991), and the radioactivity (water-extractable $^{33}P$) was measured by liquid scintillation counting (2500 TR, Packard Instrument Company Inc., Meriden, CT). Radioactive decay at each sampling time was accounted for in the $^{33}P$ calculations.

At several time points (i.e. days 3, 8, 12, 17, 21, 26 and 30; see Fig. 1), the 16 h extraction of water-extractable $^{31}P$ and $^{33}P$ was complemented with an additional 1 h extraction (Van der Paauw, 1971). All other analytical procedures remained the same.

The specific activity (kBq mg$^{-1}$ P) in the water-extractable P, as well as for microbial P and resin-extractable P, was calculated as

| Experimental phases | | Pre-incubation | Main incubation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Equilibration | | | | DRW 1 | | | | | | | | DRW 2 | | | | | | | | | DRW 3 | | | | | | | | | |
| Days before and after labeling | | -17 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| Treatments | Drying and rewetting (DRW) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Constant moisture (CM) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Labeled soils | Soil mixing | * | * | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Soil labeling | | * | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Water-extractable P | | | | * | Δ | (*) | | | Δ | (*) | | | | Δ | (*) | | Δ | (*) | | | Δ | (*) | | Δ | (*) | | | Δ | (*) | | | Δ | |
| | Microbial P | | | | * | * | (*) | | | * | (*) | * | | | * | (*) | | * | (*) | | | * | (*) | | * | (*) | | | * | (*) | | | * | |
| | P spike recovery | | | | * | * | (*) | | | * | (*) | * | | | * | (*) | | * | (*) | | | * | (*) | | * | (*) | | | * | (*) | | | * | |
| | Phosphatase activity | | | | * | * | (*) | | | * | (*) | * | | | * | (*) | | * | (*) | | | * | (*) | | * | (*) | | | * | (*) | | | * | |
| | Bacterial and fungal abundance | | | | * | * | (*) | | | * | (*) | * | | | * | (*) | | * | (*) | | | * | (*) | | * | (*) | | | * | (*) | | | * | |
| Unlabeled soils | Soil mixing | * | * | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Soil respiration | Every 2 days | | * | * | * | * | | | * | * | | | | * | * | | * | * | | | * | * | | * | * | | | * | * | | | * | |

**Fig. 1.** Schedule of performed experiments and measurements with two treatments: constant moisture (CM) and drying and rewetting (DRW) for three DRW cycles (DRW1, DRW2 and DRW3). In the treatment rows, coloured rectangles show different soil water statuses, with blue indicating moist incubations (50% of WHC, equivalent to 0.25 g g$^{-1}$ and water potential of pF 2), grey indicating drying phases (from 50% to 10% of WHC) and white indicating dry periods (10% of WHC, equivalent to 0.05 g g$^{-1}$ and water potential of pF 4.2). For each treatment, two setups were run in parallel with labelled ($^{33}P$) and unlabelled ($^{31}P$) soils. Single asterisks alone indicate the time points of manipulations and the measurements for both treatments, while the asterisks with brackets indicate the time points of measurements only for DRW treatment. With respect to water-extractable P, triangles indicate the time points when a short-time (1 h) water extraction was conducted in addition to a normal (16 h) water extraction. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$$SA_{(t)} = {}^{33}P_{(t)}/{}^{31}P_{(t)}, \tag{1}$$

where $SA_{(t)}$ is the specific activity at time t, ${}^{33}P_{(t)}$ is the radioactivity of ${}^{33}P$ in kBq per kg soil at time t and ${}^{31}P_{(t)}$ is the amount of ${}^{31}P$ per kg soil at time t in mg.

### 2.3.2. Microbial P

Microbial P was determined by liquid hexanol fumigation and simultaneous extraction with anion exchange resin membranes (Kouno et al., 1995). Moist soil equivalent to 2 g dry soil was shaken for 16 h in 30 ml $H_2O$ together with a resin membrane (BDH #55164, 6 cm × 2 cm) previously saturated with 0.5 M $NaHCO_3$. Four subsamples were included: 1) non-fumigated subsamples rendering resin-extractable P, 2) fumigated subsamples receiving 1 ml of 1-hexanol, 3) non-fumigated subsamples receiving 1 ml of a ${}^{31}P$ spike (10 µg ${}^{31}P$ per ml, which is equal to 5 mg P kg$^{-1}$ soil) and 4) non-fumigated subsamples receiving 1 ml of a ${}^{33}P$ spike (10 µg ${}^{31}P$ per ml and 6 kBq per ml, which are equal to 5 mg ${}^{31}P$ kg$^{-1}$ soil and 3 kBq g$^{-1}$ soil). After 16 h of shaking, the resin strips were rinsed with $H_2O$ and eluted with a 0.1 M NaCl/HCl solution. The concentration of ${}^{31}P$ was determined colourimetrically, and the radioactivity of ${}^{33}P$ was obtained by scintillation counting of the eluents, as outlined in section 2.3.1.

The concentration of microbial ${}^{31}P$ in mg P kg$^{-1}$ was calculated as

$$\text{microbial } {}^{31}P = ({}^{31}P_{fum} - \text{resin-extractable } {}^{31}P)/{}^{31}P_{rec}, \tag{2}$$

where ${}^{31}P_{fum}$ is the concentration of ${}^{31}P$ extracted from a fumigated subsample, resin-extractable ${}^{31}P$ is the concentration of ${}^{31}P$ from a non-fumigated subsample (both in mg P kg$^{-1}$) and ${}^{31}P_{rec}$ is the fraction of an added ${}^{31}P$ spike recovered compared with the non-fumigated subsamples.

The release of ${}^{31}P$ from microbial cells during fumigation may affect the recovery of ${}^{33}P$ from soil mineral surfaces to resin membranes, which can result in the under- or overestimation of ${}^{33}P$ contained in lysed microbial cells (McLaughlin et al., 1988). To correctly determine the amount of ${}^{33}P$ in microbial biomass, a linear relationship between ${}^{31}P$ added to subsamples 1 and 3 (0 mg P kg$^{-1}$ in non-fumigated subsample 1 and 5 mg P kg$^{-1}$ in ${}^{31}P$-spiked subsample 3) and ${}^{33}P$ recovered on the resin membranes in these subsamples was established to calculate the extracted ${}^{33}P$ (${}^{33}P_{extracted}$, in kBq g$^{-1}$) depending on the amount of ${}^{31}P$ added (in mg P kg$^{-1}$) (Bünemann et al., 2016):

$$ {}^{33}P_{extracted} = a \times {}^{31}P_{spike} + b. \tag{3}$$

The concentration of microbial ${}^{31}P$ calculated according to Equation (2) was then inserted as ${}^{31}P_{spike}$ into Equation (3). The resulting value of ${}^{33}P_{extracted}$ was inserted as resin-extractable ${}^{33}P_{corrected}$ into Equation (4) to calculate the ${}^{33}P$ that had been incorporated in microbial P (in kBq g$^{-1}$) as

$$\text{microbial } {}^{33}P = ({}^{33}P_{fum} - \text{resin-extractable } {}^{33}P_{corrected})/{}^{33}P_{rec}, \tag{4}$$

where ${}^{33}P_{fum}$ is the ${}^{33}P$ extracted from fumigated subsamples (subsample 2), and ${}^{33}P_{rec}$ is the recovery of the separate ${}^{33}P$spike (containing the same amount of ${}^{31}P$ as ${}^{31}P$ spike) from the resin membranes. The ${}^{33}P_{rec}$ was used for the time point and treatment-specific correction.

We present microbial ${}^{31}P$ and ${}^{33}P$ without the use of a conversion factor (Kp), which would account for the incomplete extraction of microbial P, as this factor is method- and soil-dependent (Oberson and Joner, 2005) and was not determined in our study.

### 2.3.3. Microbial P turnover

The microbial P turnover rate (in day$^{-1}$) was derived from the fluctuations of microbial ${}^{31}P$ during incubation (McGill et al., 1986):

$$\text{microbial P turnover rate} = \frac{\sum \text{microbial P losses}}{\text{mean microbial P} \cdot \text{days}}, \tag{5}$$

where $\Sigma_{\text{microbial P losses}}$ is the sum of the decreases in microbial ${}^{31}P$, and mean microbial P is the average microbial ${}^{31}P$. The microbial P turnover rate was calculated after the beginning of DRW1 for each day when the microbial ${}^{31}P$ was determined using microbial ${}^{31}P$ of day 0 as the starting value to compute the sum of decreases. Moreover, the microbial P turnover rate was calculated for each DRW cycle individually using the microbial P of day 3 for DRW1, day 12 for DRW2 and day 21 for DRW3 as starting values to compute the sum of decreases within each DRW cycle.

### 2.3.4. Soil abiotic and biotic indicators

Recoveries of P from soils amended with a known P-spike were determined simultaneously with the concentrations of microbial P (section 2.3.2) and used as an indicator of the soil P sorption properties.

Soil respiration was determined from unlabelled soil at each time point (Fig. 1) by trapping $CO_2$ in 10 mL of 0.1 M NaOH. After the addition of 1 ml of 1.5 M $BaCl_2$ and phenolphthalein indicator solution, back titration was performed with 0.05 M HCl (Alef, 1995).

The abundance of bacterial and fungal DNA was determined using real-time quantitative polymerase chain reaction (qPCR) analysis. Genomic soil DNA was extracted from 0.25 g of fresh weight soil at each sampling time (Fig. 1) ($n = 3$) using the PowerSoil®DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) following the manufacturer's instructions, with a slight modification in the homogenisation and lysis step using QIAGEN® Tissuelyser II for 3 min (twice) at a frequency of 30 s$^{-1}$. The extracted DNA was stored at −20 °C. The DNA sample was quantified using a DNA BR Assay Kit (Q32853) and measured on the Spark® M10 (Tecan) plate reader. Bacterial 16S rRNA gene (primers Eub338F [Lane, 1991], Eub518R [Muyzer et al., 1993]), fungal ITS (primers 5.8S [Vilgalys and Hester, 1990] and modified ITS1F [Boyle et al., 2008]) region abundances were quantified in triplicate reactions using 96-well plates on the Applied Biosystems® 7500 Fast instrument. Both bacterial and fungal qPCR reactions (25 µl) contained 2 µl 10-fold diluted template DNA, 5 µl 5 × buffer (Promega), 0.2 µM of each primer, 0.2 mM dNTPs (ThermoScientific), 1.25 µl 20 × EvaGreen (Brunschwig), 0.5 µl 50 × ROX (Invitrogen) and 0.6 U µl$^{-1}$ GoTaq DNA Polymerase (Promega). The qPCR cycling conditions were as follows (for both genes): 5 min at 95 °C, followed by 40 cycles of 60 s melt at 95 °C, 60 s annealing at 53 °C and 60 s elongation at 72 °C. A melt curve from 65 °C to 95 °C was added at the end of all amplification cycles to ensure the specificity of the reaction. Standard curves were developed in triplicate in each assay using a serial dilution of genomic DNA extracted from pure cultures (*Pseudomonas protegens* for bacteria and *Fomitopsis pinicola* for fungi). For all qPCR assays, a linear relationship was found between the log of the standard copy number and the calculated threshold cycle across the standard concentration range ($R^2 > 0.99$ in all cases).

The potential activity of phosphomonoesterase was measured on a microplate based on Marx et al.'s (2001) method as modified by Poll et al. (2006). Briefly, 1 g of soil was dispersed in 100 ml of autoclaved nanopure $H_2O$ with an ultrasonic probe for 2 min. On a magnetic stirrer plate, 50 µl of soil suspension was transferred to the microplate with six analytical replicates. 4-methylumbelliferyl-phosphate was used as the substrate, and the assay was buffered with 0.1 M MES buffer at pH 6.1 (for acid phosphatase) or with modified universal buffer at pH 11 (for alkaline phosphatase; Alef et al., 1995). After pre-incubation at 30 °C for 30 min, the increase in fluorescence was measured for 180 min (FLx800, Biotek).

### 2.4. Statistical analysis

Statistical analyses were performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL). A significant difference in the means between treatments at each sampling date was analysed using Tukey's test. The overall treatment and temporal differences were analysed by two-way ANOVA. If the F test was significant at $p < 0.01$, the least significant difference (LSD) was calculated with an alpha of 0.05. Further

details on the results of the statistical analysis are provided in Table S2.

In two of the 66 observations, the calculation according to Equation (4) gave negative microbial $^{33}P$ values for one out of the three analytical replicates, i.e. the resin-extractable $^{33}P_{corrected}$ values were higher than the recovered $^{33}P$ in the fumigated replicates. This further resulted in negative $SA_{microbial\ P}$ calculated according to Equation (1). This occurred in the first DRW cycle on days 5 and 8 when soil microbial biomass was very low, which was caused by soil dryness. As negative SAs cannot be attained and would lead to an underestimation of the average of the three replicates, we set the negative SAs of specific replicates to zero, assuming that no $^{33}P$ tracer was recovered in the microbial biomass for the respective replicate.

## 3. Results

### 3.1. Water-extractable, resin-extractable and microbial P

Water-extractable P remained constant in the CM treatment (mean of 0.4 mg kg$^{-1}$, with 1 h and 16 h extraction), but showed similar patterns of increases (up to 0.7 mg kg$^{-1}$) and decreases during each DRW cycle (Fig. 2a). Similar but weaker fluctuations were observed during DRW2 and DRW3, but an overall elevated water-extractable P level was maintained in the DRW treatment. A shorter extraction time of 1 h was yielded by tendency higher water-extractable P values during dryness and lower values during rewetting. Resin-extractable P (Fig. 2b) increased in both treatments after day 1 (4.0 mg kg$^{-1}$) but then remained constant in the CM treatment (4.6 mg kg$^{-1}$). For the DRW treatment, only on day 8 (DRW1) we observed a significant increase in resin P (5.8 mg kg$^{-1}$), but no differences between CM and DRW were found for other time points. Microbial P fluctuated slightly in the CM treatment, with a mean of 3.6 mg kg$^{-1}$ over the entire incubation period (Fig. 2c). During DRW1, microbial P was significantly reduced by half, but recovered quickly during the subsequent rewetting. Fluctuations in microbial P became weaker in later DRW cycles and remained almost

constant and similar to those in CM during DRW3.

The $SA_{water-extractable\ P}$ continuously decreased from 400 to 200 kBq mg$^{-1}$ P during the 30 days of soil incubation in the CM treatment (Fig. 2d). Slightly yet significantly elevated values were obtained in the DRW treatment, except day 8 (DRW1) when $SA_{water-extractable\ P}$ in the 1 h extraction peaked at 470 kBq mg$^{-1}$ P. Similarly, $SA_{resin-extractable\ P}$ decreased from 570 to 300 kBq mg$^{-1}$ P during the 30 days of soil incubation, with the DRW treatment having slightly elevated values compared to the CM treatment on most days (Fig. 2e). The $SA_{microbial\ P}$ (Fig. 2f) in the CM treatment was 200–270 kBq mg$^{-1}$ P with no temporal trend (except for day 12; 103 kBq mg$^{-1}$ P). In DRW1, $SA_{microbial\ P}$ already significantly decreased during the moist phase (day 3) and reached 120 kBq mg$^{-1}$ P during soil drying of DRW1, followed by a quick recovery to initial values by rewetting. During DRW2, $SA_{microbial\ P}$ increased to 285 kBq mg$^{-1}$ P but afterwards remained constant and similar to the values of the CM treatment.

### 3.2. Indicator of abiotic P dynamics

Soil P sorption capacity was low, given a $^{31}P$ or $^{33}P$ spike recovery on anion exchange resin membranes from soil suspensions of 60% or higher (Fig. 3a, b, c). The P-spike recovery was consistently and significantly higher in the DRW treatment than in CM on all days, indicating a decrease in soil P sorption during DRW, which was maintained in the soil for the rest of the soil incubation.

### 3.3. Indicators of biotic P dynamics

The rate of microbial P turnover derived from the fluctuations of microbial $^{31}P$ was higher under DRW than under CM conditions (Fig. 4). In DRW, the microbial $^{31}P$ turnover rate was 0.14 day$^{-1}$ on day 5 (dryness period) and then gradually decreased with time but remained consistently elevated compared with that in the CM treatment. The same pattern appeared when calculating the microbial P turnover times for
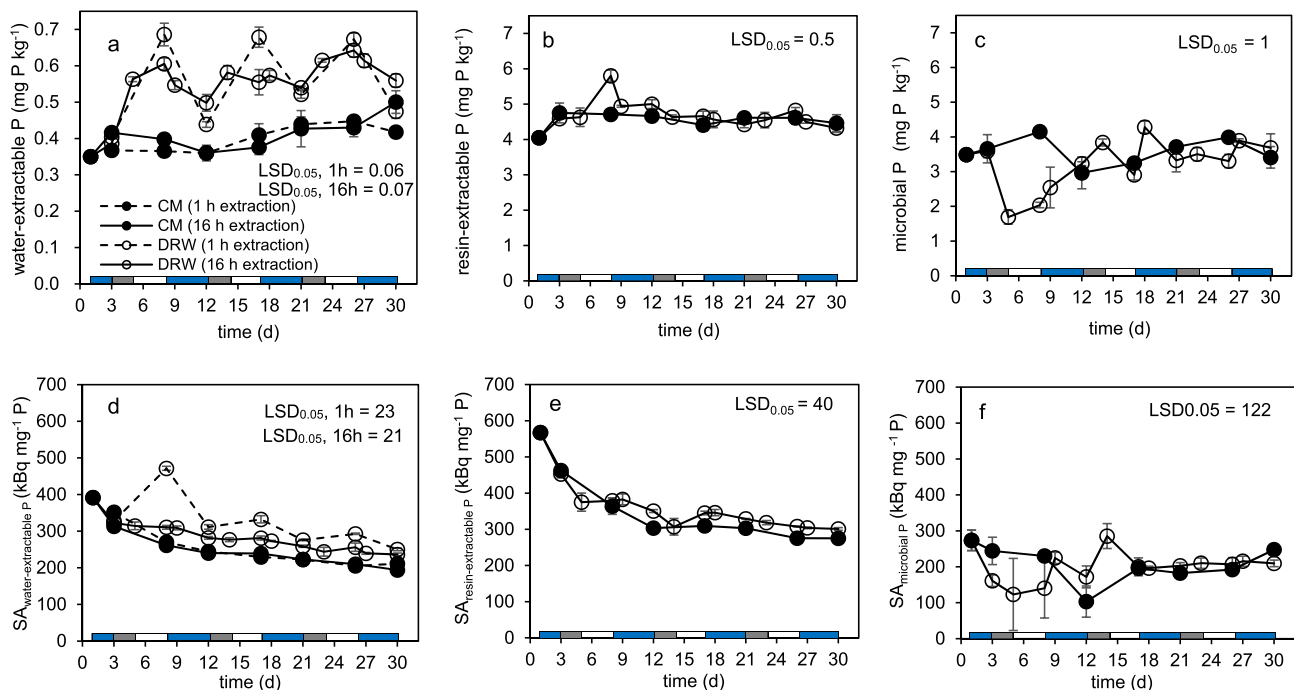


**Fig. 2.** Concentration of $^{31}P$ (a, b, c) and specific activity (d, e, f) of water-extractable P (a, d), resin-extractable P (b, e) and microbial P (c, f) in incubated soils from constant moisture (CM) and drying and rewetting (DRW) treatments. Data points are connected by lines to visualise the trend. Day 0 refers to the day of soil labelling with $^{33}P$. Error bars indicate the standard error of the three replicates. LSD refers to the least significant difference between all data shown. For Figs. a and d, the treatment comparisons of 1 h and 16 h extractions are separate. Coloured rectangles show different soil water statuses in the DRW treatment during incubation. They are not shown for the CM treatment, as the soil water content was constant (see details in Fig. 1).
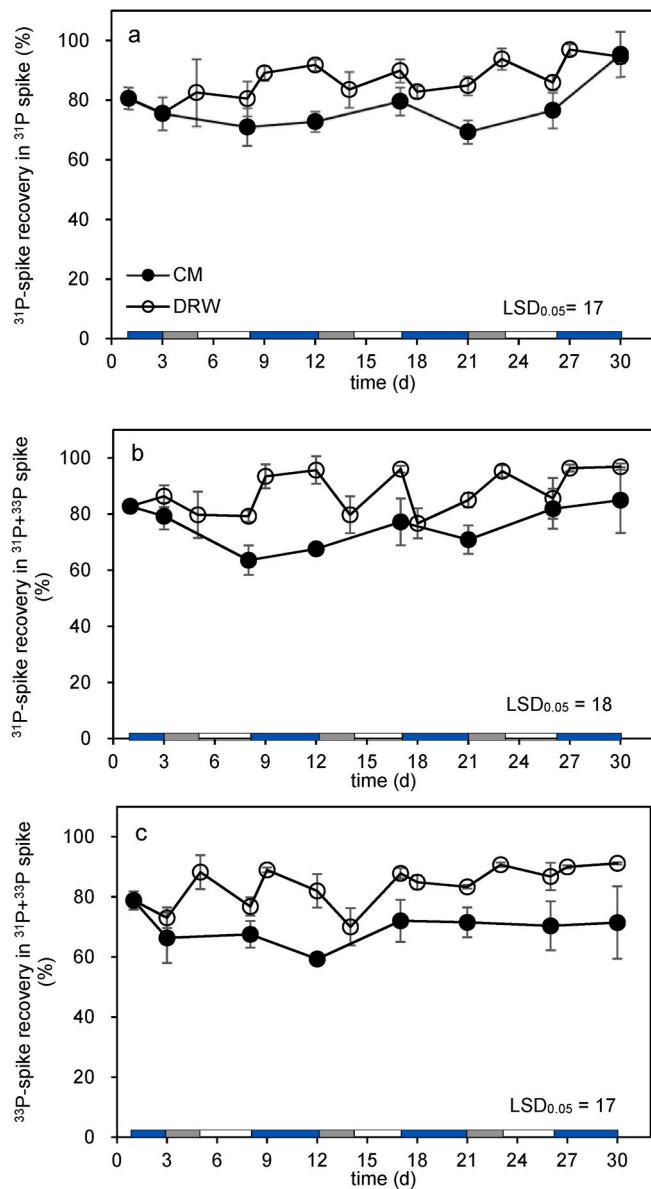
**Fig. 3.** Recoveries of an added $^{31}$P or $^{33}$P spike to soil as an indicator of the soil's P sorption properties. Two different P spikes were used ($^{31}$P alone or $^{31}$P in combination with a $^{33}$P spike). Data points are connected by lines to visualise the trend. Day 0 refers to the day of soil labelling with $^{33}$P. Error bars indicate the standard error of the three replicates. LSD refers to the least significant difference between all data shown.

each DRW cycle individually, indicating that microbial P turnover was reduced with an increasing number of DRW cycles (DRW1: 0.09 d$^{-1}$, DRW2: 0.06 d$^{-1}$, DRW3: 0.03 d$^{-1}$).

Soil respiration was constant during the last days of pre-incubation and sharply increased on day 0 as an effect of soil labelling by mixing (Fig. 5a). In CM, the respiration rates decreased again with time, reaching similar rates as those during pre-incubation on day 12 and then remained constant. In the DRW treatment, soil drying (days 3–5, 12–14 and 21–23) and rewetting (days 9, 18 and 27) caused peaks of increased soil respiration, while respiration rates were low at the end of each dry phase (days 8, 17 and 26). The fluctuations in respiration rate decreased with increasing numbers of DRW cycles. The cumulative respiration at the end of the incubation was 360 mg C kg$^{-1}$ and was the same for both treatments (Fig. 5b).

Bacterial gene copies declined with time in both treatments, and fungal gene copies showed more fluctuations independent of DRW
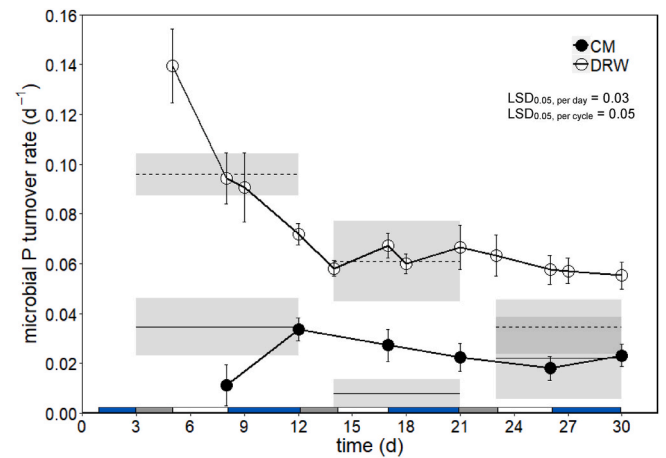


**Fig. 4.** Microbial P turnover rates derived from fluctuations of microbial $^{31}$P calculated either for each given day (circles with connecting lines to visualise the trend) or for each DRW cycle individually (full horizontal [CM] and dashed horizontal [DRW] lines with grey areas indicate the respective standard error). The LSD refers to the least significant difference between all data shown (the treatment comparisons per day and per cycle are separate).
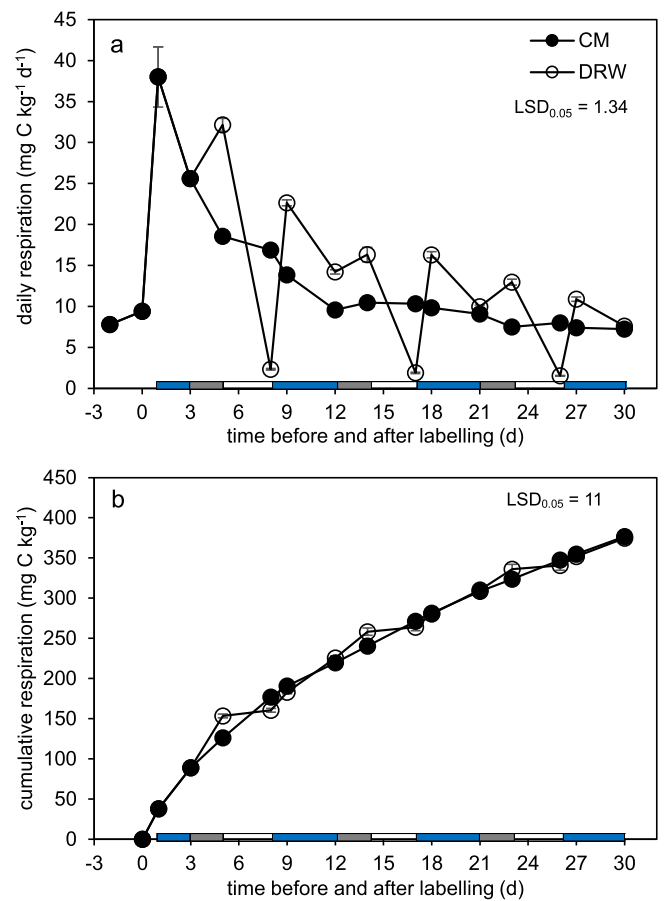


**Fig. 5.** Daily soil respiration rates (a) and cumulative soil respiration (b) of soils treated with constant moisture (CM) and drying and rewetting (DRW) during pre-incubation (time before labelling on day 0) and during the main experiment (time after labelling). Data points are connected by lines to visualise the trend. Day 0 refers to the day of soil labelling with $^{33}$P. Error bars indicate the standard error of the three replicates (smaller than the symbol in most cases). LSD refers to the least significant difference between all data shown.

treatment (Fig. 6a and b). Bacterial and fungal gene copies were often higher in CM than in DRW, with particularly large differences on day 17 for bacteria and days 8 and 12 for fungi (Fig. 6a and b). The strong abundance of bacteria compared with fungi was indicated by a bacteria: fungi ratio of 22 at the beginning of incubation (Fig. 6c). Except for DRW1, the bacteria: fungi ratio remained similar for both treatments and continuously became narrower during the course of the experiment.

The potential activity of acid phosphomonoesterases was approximately six times higher than that of alkaline phosphomonoesterases (Fig. 7a and b). The potential activities of both phosphomonoesterases were at most time points higher and significantly elevated in the DRW compared to the CM treatment.
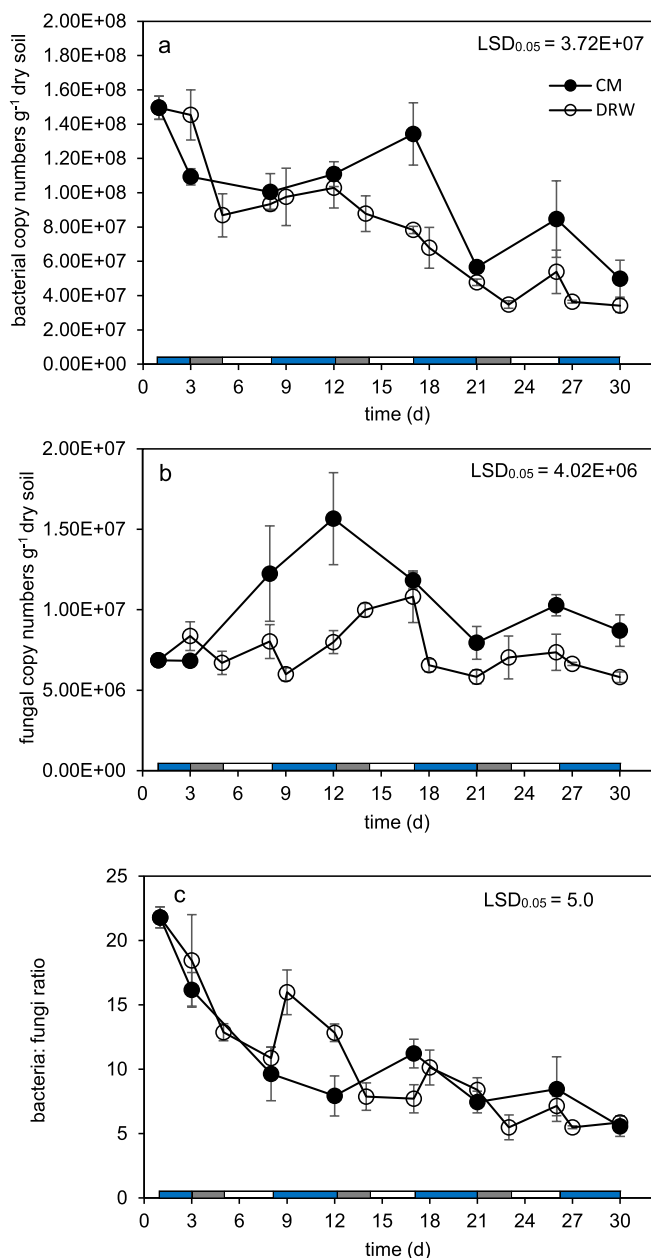


**Fig. 6.** Abundance of bacteria (a) and fungi (b) and their ratios in incubated soils from constant moisture (CM) and drying and rewetting (DRW) treatments. Data points are connected by lines to visualise the trend. Day 0 refers to the day of soil labelling with $^{33}$P. Error bars indicate the standard error of the three replicates. LSD refers to the least significant difference between all data shown.
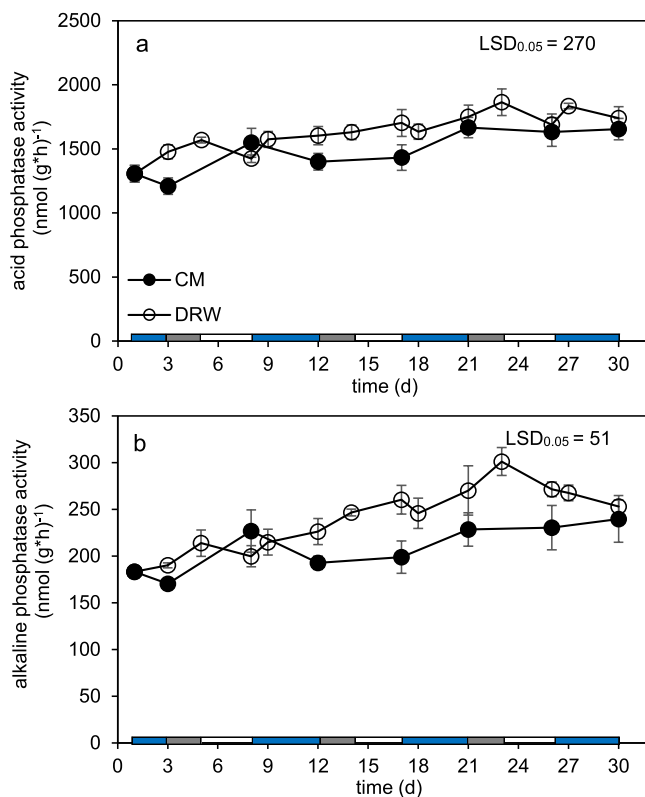


**Fig. 7.** Potential acid (a) and alkaline (b) phosphomonoesterase activities in incubated soils from constant moisture (CM) and drying and rewetting (DRW) treatments. Data points are connected by lines to visualise the trend. Day 0 refers to the day of soil labelling with $^{33}$P. Error bars indicate the standard error of the three replicates. LSD refers to the least significant difference between all data shown.

## 4. Discussion

### 4.1. Dynamics of water-extractable P, resin-extractable P and P sorption in soil under repeated DRW

Water-extractable P was most intensively affected by the repeated DRW regime, whereas resin-extractable P and microbial P showed responses during DRW1 or DRW2, but were latest by DRW3 unresponsive to the DRW treatment (Fig. 2). Although drying induced an increase in water-extractable P in DRW1 (0.2 mg P kg$^{-1}$ soil, Fig. 2a), which coincided with the decrease in microbial P (2 mg P kg$^{-1}$ soil, Fig. 2c), only one-tenth of the microbial P released during DRW was recovered in water-extractable P. Moreover, the shorter 1 h extraction for water-extractable P (allowing less time for soil P sorption) or the more effective extraction by resins did not result in a full recovery of microbial P released during drying in DRW1. This means that a significant fraction of the microbial P released during DRW 1 was incorporated into a soil P fraction, which was more strongly bound to the soil solid phase. Indeed, the increases in P-spike recoveries (Fig. 3) indicate that the microbial P released during DRW became and remained adsorbed on the soil solid phase for the rest of the soil incubation period, thus decreasing the overall soil P sorption properties. Therefore, it appears that the elevated water-extractable P concentrations in response to DRW were not only directly caused by the release of microbial P upon DRW but were also indirectly supported by a decrease in soil P sorption properties, allowing for higher water-extractable P concentrations to sustain. Given the elevated water-extractable P values throughout DRW2 and DRW3 (Fig. 2a) along with constantly elevated P-spike recoveries (Fig. 3), this indirect effect appears to be more long-lasting than the direct effect. The DRW-induced changes in soil P sorption likely play a greater role in soils

with a larger number of P sorption sites, as indicated by the strong differences in DRW-induced P leaching losses in soils with high versus low iron and aluminium oxides, which serve as P sorption sites (Lang et al., 2017; Brödlin et al., 2018).

### 4.2. Microbial P dynamics altered by DRW

The indicators in the biotic activity of the soil most strongly responded to DRW1, whereas the later DRW cycles had little to no effect on soil respiration rates (Fig. 5), microbial P (Fig. 2c), the calculated microbial P turnover times (Fig. 4) or the abundance of bacteria and fungi in the soil (Fig. 6), except for potential phosphatase activities (Fig. 7). Whereas the reduction of microbial P by half in DRW1 likely had a substantial effect on the overall microbial biomass, the reduction in pool size (i.e. microbial P) should have no effect on the $SA_{microbial\ P}$, as $^{31}P$ and $^{33}P$ should be equally affected. However, during DRW1, we observed a decrease in $SA_{microbial\ P}$ to an almost similar proportion as microbial P, which could be interpreted in two ways:

a) Specific microorganisms took up (i.e. immobilised) a disproportionately high fraction of the $^{33}P$ isotope during soil labelling, whereas other microorganisms immobilised the $^{33}P$ isotope at a slower rate. Simultaneously, the microorganisms characterised by a more rapid $^{33}P$ uptake were more susceptible to soil drying, causing a disproportionate dilution of the microbial P pool through microbial die-off upon soil drying.
b) Microorganisms immobilised P with a lower SA, thus diluting the SA of the microbial P pool. As both water-extractable P and resin-extractable P had a similar or higher SA than the $SA_{microbial\ P}$ at all times, this newly immobilised P could have originated from unlabelled soil organic P.

Both of the suggested interpretations were equally plausible and supported by our dataset. Although we assume that both processes potentially took place simultaneously (but probably to different extents), we discuss each process individually in the following sections.

Concerning the preferential uptake of $^{33}P$ isotope in combination with higher susceptibility towards the drying of specific soil microorganisms, considering soil microbial P as a homogeneous pool is most likely not adequate, given the high microbial diversity in soils. For example, Achat et al. (2010) suggested two microbial P pools with different turnover times in soil. Bacteria, particularly gram-negative bacteria (Van Gestel et al., 1993), are often characterised as fast growers because they are quick to colonise and use labile C sources (Reischke et al., 2014), whereas fungi often use more recalcitrant C sources (Brant et al., 2006). Moreover, fungi were more likely affected by the induced soil disturbance by mixing that was necessary to guarantee a homogenous distribution of $^{33}P$ label in the soil, an effect similar to soil ploughing, which disrupts hyphal networks in soil (Kabir et al., 1998). This is supported by the higher bacteria: fungi ratio at the beginning of the soil incubation (22, day 1), as it was reduced within the first seven days to a bacteria: fungi ratio of around 8 during incubation in both treatments (Fig. 6). This suggests that bacteria could have potentially immobilised a higher fraction of added $^{33}P$ isotope, not only because of their higher abundance, but also because of their ability for rapid resource acquisition (relative $^{33}P$ enrichment compared with fungi). However, given a generally higher susceptibility of (gram-negative) bacteria than fungi to drought (Dinh et al., 2017; de Vries et al., 2018; Hicks et al., 2019), the decrease in microbial P during DRW1 (Fig. 2c) could have affected those bacteria, causing a relative depletion of $^{33}P$ in bacteria compared with fungi. Notably, the bacteria: fungi ratio was not different between the treatments until day 8 (end of dryness DRW1), and the largest decreases in $SA_{microbial\ P}$ were observed between day 1 until day 8. This suggests that the microbial community most involved in this process did not play a quantitatively relevant role in the DNA formation of the microbial P pool or that this process was minor compared with the increased mineralisation of soil organic P.

Concerning the mineralisation and microbial immobilisation of unlabelled organic P causing the decrease in $SA_{microbial\ P}$, the newly immobilised P must have originated from a source that was not available for microbial P uptake before DRW1. Barnard et al. (2020) recently suggested that previously unavailable organic matter could become available only as a consequence of soil drying if a certain threshold of rewetting-induced upshock was passed. This pre-condition enabling soil microorganism to mineralise previously unlabelled organic P was probably met in the intense DRW regime of our study (Fig. S1). Interestingly, we observed peaks of soil respiration during each of the three periods of soil drying (Fig. 5a), whereas soil microbial activity and soil respiration were previously reported to generally decline during the soil drying period (Schimel, 2018). Thus, we interpret the peaks in soil respiration during soil drying as being not part of the Birch effect, which describes the pulse of soil respiration after rewetting dried soils (Birch, 1958). Along with the observed decrease in $SA_{microbial\ P}$ during drying of DRW1, we instead interpret the peaks in soil respiration during soil drying as the mineralisation of soil organic matter with lower or no SA, which was unavailable for microbial mineralisation and immobilisation prior to the drying event. Specifically, during the first drying in DRW1 until day 5, an additional 28 mg C kg$^{-1}$ was released as $CO_2$ in DRW compared with CM (Fig. 5). This amount could be assumed to be only 60% of the actual mineralised C released as $CO_2$, while 40% was assimilated in the newly formed microbial biomass (Murphy et al., 2003; Achat et al., 2012a). Assuming that the mineralised substrate had either a) a C: P ratio similar to the one of the microbial biomass of this soil (i.e. 16; Chen et al., 2018) or b) a C: organic P ratio of the soil organic matter (i.e. 120; Table 1), about 3 mg P kg$^{-1}$ soil (assumption a) or 0.4 mg P kg$^{-1}$ soil (assumption b) of organic P would have been additionally mineralised during first drying in DRW1, which would be directly available for microbial P immobilisation, but with a substantially lower (or no) SA. Our hypothesis of additional organic P made available during DRW is corroborated by Sun et al. (2017), reporting on increased concentrations of organic P in soil bicarbonate extracts after short term soil drying. As different soil phosphatase enzymes are mostly sorbed to the soil solid phase (Jarosch et al., 2019), and an increase in potential phosphomonoesterase activities in the DRW treatment was observed (Fig. 7), an increased organic P mineralisation despite reduced availability of water is supported. Although physical soil disaggregation upon drying could also render previously unlabelled inorganic P available for microbial immobilisation (Sinaj et al., 1997), an increase in unlabelled inorganic P could also result in decreased $SA_{water-extractable\ P}$. However, this was not observed in our study (Fig. 2d), supporting our assumption that DRW caused the release of previously unavailable organic P, which became mineralised and immobilised by microorganisms.

The comparison between $SA_{resin-extractable\ P}$ and $SA_{microbial\ P}$ suggests that resin-extractable inorganic P was an important source of microbial P uptake under both CM and DRW treatments. After 30 days, the $SA_{microbial\ P}$ was between 70% (DRW) and 90% (CM) of $SA_{resin-extractable\ P}$ (Fig. 2e and f), suggesting that 70%–90% of microbial P was derived from resin-extractable P. However, with rapid microbial turnover, for example, a microbial P turnover time of 11 days during DRW1 (Fig. 4), the SA of the resin-extractable P itself was affected by microbial biomass P. As all three studied pools, namely water-extractable P, resin-extractable P and microbial P, had similar SA at the end of the incubation, the P transfer between these pools could not be deduced but showed their close connection.

### 4.3. Temporal effect of DRW on soil P dynamics

Using a sandy soil from a permanent grassland as the model system, we found that only water-extractable P (Fig. 2a), soil P sorption (Fig. 3) and potential phosphatase activities (Fig. 7a and b) remained altered after three DRW cycles, whereas the other soil P pools and indicators of soil P dynamics were not affected latest by DRW3. Notably, microbial P

became increasingly adjusted to DRW (Fig. 2c), resulting in a reduced turnover of microbial P in later DRW cycles (Fig. 4). The weaker effects on microbial P during repeated DRW have also been previously reported (Chen et al., 2016), suggesting an active selection induced by DRW1 for microorganisms that are more adjusted to the re-occurrence of DRW. The slacking of soil aggregates was reported to be reduced in the later DRW cycles of soils (Denef et al., 2001). Therefore, the additional release of organic matter for microbial mineralisation and immobilisation, as well as the additional exposure of soil P sorption sites, is likely to be limited in later shortly repeated DRW cycles.

## 5. Conclusion

Using a sandy soil, we simulated three DRW cycles of nine days each to determine the effect on soil P dynamics by determining the soil P pool changes and recovery of an applied isotopic $^{33}$P tracer. The first DRW cycle had the largest effect on microbial P (reduction by half), but water-extractable P and resin-extractable P did not increase proportionally. Instead, soil P sorption decreased and remained reduced for the rest of the incubation, indicating that the released microbial P became adsorbed and that soil water-extractable P was indirectly increased by the reduced P sorption. The reduction in the specific activity of microbial P in the first DRW cycle was interpreted as a combination of two processes: a) the quick incorporation of P and isotopic $^{33}$P tracer and the higher susceptibility of specific soil microorganisms to soil drying and b) the increased mineralisation of previously unavailable soil organic P compounds. As similar patterns were not detected in the subsequent DRW cycles, microbial P became adjusted to the repeated DRW cycles. This was supported by the microbial P turnover times and the abundance of bacteria and fungi, which were also indifferent to the CM-control treatment. We conclude that the current concepts suggesting an increased mobility of soil P towards other environmental compartments due to soil DRW should consider the adjustment of soils to this environmental stress in case of short repetition of DRW incidences. Determining how microbial P and other indicators of biotic P dynamics can develop with a longer experimental duration and in soils with different properties requires further testing.

## Declaration of competing interest

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

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2020.108079.

## References

Achat, D.L., Augusto, L., Bakker, M.R., Gallet-Budynek, A., Morel, C., 2012a. Microbial processes controlling P availability in forest spodosols as affected by soil depth and soil properties. Soil Biology and Biochemistry 44, 39–48.

Achat, D.L., Augusto, L., Gallet-Budynek, A., Bakker, M.R., 2012b. Drying-induced changes in phosphorus status of soils with contrasting soil organic matter contents-implications for laboratory approaches. Geoderma 187, 41–48.

Achat, D.L., Morel, C., Bakker, M.R., Augusto, L., Pellerin, S., Gallet-Budynek, A., Gonzalez, M., 2010. Assessing turnover of microbial biomass phosphorus: combination of an isotopic dilution method with a mass balance model. Soil Biology and Biochemistry 42, 2231–2240.

Alef, K., 1995. Soil respiration. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 214–219.

Anderson, J.M., Ingram, J.S.I., 1993. Tropical Soil Biology and Fertility. A Handbook of Methods. CAB International, Wallingford, Oxon, UK, p. 221.

Barnard, R.L., Blazewicz, S.J., Firestone, M.K., 2020. Rewetting of soil: revisiting the origin of soil $CO_2$ emissions. Soil Biology and Biochemistry 147, 107819.

Birch, H., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9–31.

Blackwell, M.S.A., Brookes, P.C., de la Fuente-Martinez, N., Gordon, H., Murray, P.J., Snars, K.E., Williams, J.K., Bol, R., Haygarth, P.M., 2010. Phosphorus solubilization and potential transfer to surface waters from the soil microbial biomass following drying–rewetting and freezing–thawing. Advances in Agronomy 106, 1–35.

Boyle, S.A., Yarwood, R.R., Bottomley, P.J., Myrold, D.D., 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology and Biochemistry 40, 443–451.

Brant, J.B., Sulzman, E.W., Myrold, D.D., 2006. Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation. Soil Biology and Biochemistry 38, 2219–2232.

Bremner, J.M., 1965. Determination of nitrogen in soil by the Kjeldahl method. Journal of Agricultural Science 55, 11–33.

Brödlin, D., Kaiser, K., Kessler, A., Hagedorn, F., 2018. Drying and rewetting foster phosphorus depletion of forest soils. Soil Biology and Biochemistry 128, 22–34.

Bünemann, E.K., Augstburger, S., Frossard, E., 2016. Dominance of either physicochemical or biological phosphorus cycling processes in temperate forest soils of contrasting phosphate availability. Soil Biology and Biochemistry 101, 85–95.

Bünemann, E.K., Keller, B., Hoop, D., Jud, K., Boivin, P., Frossard, E., 2013. Increased availability of phosphorus after drying and rewetting of a grassland soil: processes and plant use. Plant and Soil 370, 511–526.

Bünemann, E.K., Oberson, A., Liebisch, F., Liebisch, F., Annaheim, K.E., Huguenin-Elie, O., Frossard, E., 2012. Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. Soil Biology and Biochemistry 51, 84–95.

Butterly, C.R., Bünemann, E.K., McNeill, A.M., Baldock, J.A., Marschner, P., 2009. Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. Soil Biology and Biochemistry 41, 1406–1416.

Cade-Menun, B.J., Preston, C.M., 1996. A comparison of soil extraction procedures for $^{31}$P NMR spectroscopy. Soil Science 161, 770–785.

Chen, C.R., Condron, L.M., Davis, M.R., Sherlock, R.R., 2003. Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. Forest Ecology and Management 177, 539–557.

Chen, H., Lai, L., Zhao, X., Li, G., Lin, Q., 2016. Soil microbial biomass carbon and phosphorus as affected by frequent drying-rewetting. Soil Research 54, 321–327.

Chen, H., Zhao, X., Chen, X., Lin, Q., Li, G., 2018. Seasonal changes of soil microbial C, N, P and associated nutrient dynamics in a semiarid grassland of north China. Applied Soil Ecology 128, 89–97.

Chepkwony, C.K., Haynes, R.J., Swift, R.S., Harrison, R., 2001. Mineralization of soil organic P induced by drying and rewetting as a source of plant-available P in limed and unlimed samples of an acid soil. Plant and Soil 234, 83–90.

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology and Biochemistry 33, 1599–1611.

de Vries, F.T., Griffiths, R.I., Bailey, M., Craig, H., Girlanda, M., Gweon, H.S., Hallin, S., Kaisermann, A., Keith, A.M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N., Prosser, J.I., Thion, C., Thomson, B., Bardgett, R.D., 2018. Soil bacterial networks are less stable under drought than fungal networks. Nature Communications 9, 3033.

Dinh, M.-V., Guhr, A., Spohn, M., Matzner, E., 2017. Release of phosphorus from soil bacterial and fungal biomass following drying/rewetting. Soil Biology and Biochemistry 110, 1–7.

Forber, K.J., Withers, P.J.A., Ockenden, M.C., Haygarth, P.M., 2018. The phosphorus transfer continuum: a framework for exploring effects of climate change. Agric. Environ. Lett. 3, 180036.

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C., 2000. Processes governing phosphorus availability in temperate soils. Journal of Environmental Quality 29, 15–23.

Haynes, R., Swift, R., 1985a. Effects of air-drying on the adsorption and desorption of phosphate and levels of extractable phosphate in a group of acid soils, New Zealand. Geoderma 35, 145–157.

Haynes, R., Swift, R., 1985b. Effect of liming and air-drying on the adsorption of phosphate by some acid soils. Journal of soil science 36, 513–521.

Hicks, L.C., Ang, R., Leizeaga, A., Rousk, J., 2019. Bacteria constrain the fungal growth response to drying-rewetting. Soil Biology and Biochemistry 134, 108–112.

Hömberg, A., Matzner, E., 2018. Effects of drying and rewetting on soluble phosphorus and nitrogen in forest floors: an experiment with undisturbed columns. Journal of Plant Nutrition and Soil Science 181, 177–184.

IPCC, 2014. Climate change 2014: synthesis report. In: core writing team. In: Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, p. 151.

ISSS Working Group RB, 1998. In: Bridges, E.M., et al. (Eds.), World Reference Base for Soil Resources. Atlas, first ed. ISSS/ISRIC/FAO. Acco, Leuven, Belgium.

Jarosch, K.A., Kandeler, E., Frossard, E., Bünemann, E.K., 2019. Is the enzymatic hydrolysis of soil organic phosphorus compounds limited by enzyme or substrate availability? Soil Biology and Biochemistry 139, 107628.

Kabir, Z., O'Halloran, I., Widden, P., Hamel, C., 1998. Vertical distribution of arbuscular mycorrhizal fungi under corn (Zea mays L.) in no-till and conventional tillage systems. Mycorrhiza 8, 53–55.

Kalembasa, S.J., Jenkinson, D.S., 1973. Comparative study of titrimetric and gravimetric methods for determination of organic carbon in soil. Journal of the Science of Food and Agriculture 24, 1085–1090.

Kouno, K., Tuchiya, Y., Ando, T., 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biology and Biochemistry 27, 1353–1357.

Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematic. John Wiley & Sons, Chichester, United Kingdom, pp. 115–175.

Lang, F., Krüger, J., Amelung, W., Willbold, S., Frossard, E., Bünemann, E., Bauhus, J., Nitschke, R., Kandeler, E., Marhan, S., 2017. Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. Biogeochemistry 136, 5–29.

Liebisch, F., Keller, F., Huguenin-Elie, O., Frossard, E., Oberson, A., Bünemann, E.K., 2014. Seasonal dynamics and turnover of microbial phosphorus in a permanent grassland. Biology and Fertility of Soils 50, 465–475.

Marx, M.C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in sols. Soil Biology and Biochemistry 33, 1633–1640.

McGill, W.B., Cannon, K.R., Robertson, J.A., Cook, F.D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. Canadian Journal of Soil Science 66, 1–19.

McLaughlin, M.J., Alston, A.M., Martin, J.K., 1988. Phosphorus cycling in wheat-pasture rotations. II. The role of the microbial biomass in phosphorus cycling. Australian Journal of Soil Research 26, 333–342.

Miller, R.O., Kotuby-Amacher, J., Rodriguez, J.B., 1997. The measurement of soil pH in a soil-water saturated paste. OMAFRA accredited method. In: Western States Laboratory Proficiency Testing Program Soil and Plant Analytical Methods, vol. 4, p. 15. Version.

Murphy, D.V., Recous, S., Stockdale, E.A., Fillery, I.R.P., Jensen, L.S., Hatch, D.J., Goulding, K.W.T., 2003. Gross nitrogen fluxes in soil: theory, measurement and application of $^{15}$N pool dilution techniques. Advances in Agronomy 79, 69–118.

Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695–700.

Oberson, A., Joner, E.J., 2005. Microbial turnover of phosphorus in soil. In: Turner, B.L., Frossard, E., Baldwin, D.S. (Eds.), Organic Phosphorus in the Environment. CABI Publishing, Cambridge, pp. 133–164.

Oberson, A., Friesen, D.K., Rao, I.M., Bühler, S., Frossard, E., 2001. Phosphorus transformations in an Oxisol under contrasting land-use systems: the role of the soil microbial biomass. Plant and Soil 237, 197–210.

Ohno, R., Zibilske, L.M., 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Science Society of America Journal 55, 892–895.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture Circular 939, 1–19.

Olsen, R., Court, M., 1982. Effect of wetting and drying of soils on phosphate adsorption and resin extraction of soil phosphate. Journal of Soil Science 33, 709–717.

Preece, C., Verbruggen, E., Liu, L., Weedon, J.T., Peñuelas, J., 2019. Effects of past and current drought on the composition and diversity of soil microbial communities. Soil Biology and Biochemistry 131, 28–39.

Poll, C., Ingwersen, J., Stemmer, M., Gerzabek, M.H., Kandeler, E., 2006. Mechanisms of solute transport affect small-scale abundance and function of soil microorganisms in the detritusphere. European Journal of Soil Science 57, 583–595.

Richardson, A.E., Simpson, R.J., 2011. Soil microorganisms mediating phosphorus availability. Plant Physiology 156, 989–996.

Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biology and Biochemistry 70, 88–95.

Schimel, J.P., 2018. Life in Dry Soils: effects of drought on soil microbial communities and processes. Annual Review of Ecology, Evolution, and Systematics 49, 409–432.

Schneider, K.D., Voroney, R.P., Lynch, D.H., Oberson, A., Frossard, E., Bünemann, E.K., 2017. Microbially-mediated P fluxes in calcareous soils as a function of water-extractable phosphate. Soil Biology and Biochemistry 106, 51–60.

Sheldrick, B.H., Wang, C., 1993. Particle size distribution. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. CRC Press, Boca Raton, FL, USA, pp. 499–507.

Sinaj, S., Frossard, E., Fardeau, J.C., 1997. Isotopically exchangeable phosphate in size fractionated and unfractionated soil. Soil Science Society of America Journal 61, 1413–1417.

Speir, T., Ross, D., 1981. A comparison of the effects of air-drying and acetone dehydration on soil enzyme activities. Soil Biology and Biochemistry 13, 225–229.

Sun, D., Bi, Q., Xu, H., Li, K., Liu, X., Zhu, J., Zhang, Q., Jin, C., Lu, L., Lin, X., 2017. Degree of short-term drying before rewetting regulates the bicarbonate-extractable and enzymatically hydrolyzable soil phosphorus fractions. Geoderma 305, 136–143.

Turner, B.L., Haygarth, P.M., 2001. Phosphorus solubilization in rewetted soils. Nature 411, 258.

Van der Paauw, F., 1971. An effective water extraction method for the determination of plant-available soil phosphorus. Plant and Soil 34, 467–481.

Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass responses to soil drying and rewetting-the fate of fast-growing and slow-growing microorganisms in soils from different climates. Soil Biology and Biochemistry 25, 109–123.

Vilgalys, R., Hester, M., 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172, 4238–4246.

Yevdokimov, I., Larionova, A., Blagodatskaya, E., 2016. Microbial immobilization of phosphorus in soils exposed to drying-rewetting and freeze-thawing cycles. Biology and Fertility of Soils 52, 685–696.

Yli-Halla, M., Schick, J., Kratz, S., Schnug, E., 2016. Determination of plant available P in soil. In: Schnug, E., De Kok, L.J. (Eds.), Phosphorus in Agriculture: 100% Zero. Springer, pp. 63–93.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Arcenegui, V., García-Orenes, F., Mataix-Beneyto, J., 2006. Assessing air-drying and rewetting pre-treatment effect on some soil enzyme activities under Mediterranean conditions. Soil Biology and Biochemistry 38, 2125–2134.