

Seasonal variability of soil phosphate stable oxygen isotopes in rainfall manipulation experiments

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Received 30 August 2010; accepted in revised form 28 April 2011; available online 6 May 2011

Abstract

Phosphorus (P) availability limits productivity in many ecosystems worldwide. As a result, improved understanding of P cycling through soil and plants is much desirable. The use of the oxygen isotopes associated to phosphate can be used to study the cycle of P in terrestrial systems. However, changes with time in the oxygen isotopes associated to available P have not yet been evaluated under field conditions. Here we present the variations in available-P oxygen isotopes, based on resin extractions, in a semi-arid site that included plots in which the amount of rainfall reaching the soil was modified. In addition, the oxygen isotopes in the less dynamic fraction which is extractable by HCl, were also measured. The $\delta^{18}\text{O}$ of the HCl-extractable phosphate shows no seasonal pattern and corresponds to the average value of the available phosphate of 16.5‰. This value is in the expected range for equilibration with soil water at the prevailing temperatures in the site. The $\delta^{18}\text{O}$ values of resin-extractable P showed a range of 14.5–19.1‰ (SMOW), and evidence of seasonal variability, as well as variability induced by rainfall manipulation experiments. We present a framework for analyzing the isotopic ratios in soil phosphate and explain the variability as mainly driven by phosphate equilibration with soil water, and by the isotopic effects associated with extracellular mineralization. Additional isotopic effects result from fractionation in uptake, and the input to the soil of phosphate equilibrated in leaves. These results suggest that the $\delta^{18}\text{O}$ of resin-extractable P is an interesting marker for the rate of biological P transformations in soil systems.

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1. INTRODUCTION

Phosphorus (P) is an essential macronutrient for living organisms. It is a constituent of major bio-molecules such as ATP, RNA, DNA, and phospholipids. The main source of P to natural terrestrial ecosystem is the soil parent material, although supply by dust can make significant contribution in some tropical and semiarid ecosystems (Swap et al., 1992; Okin et al., 2004; Mahowald et al., 2008). In natural systems, a large fraction of the P is recycled within the eco-

system (Schlesinger, 1991), while the remainder is lost to rivers and groundwater.

Phosphorus is by far the least mobile macronutrient under most soil conditions (Schlesinger, 1991; Hinsinger, 2001). The poor mobility of soil phosphate is due to the incorporation of organic P in stable compounds and to the sorption of the inorganic fraction by numerous soil constituents, such as iron and aluminum oxides in highly weathered tropical soils (Ae et al., 1990; Batjes and Sombroek, 1997), and oxides and calcium in calcareous soils (Schlesinger, 1991). This makes most of the soil phosphate scarcely available for plants, resulting in P limitation to growth.

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Tracing the biogeochemical cycling of P in soils is challenging in comparison to other macronutrients (C, N, and S). One reason is that in contrast to these other macronutrients, phosphorus has only one stable isotope – ^{31}P . Phosphorus has two radioactive isotopes (^{32}P and ^{33}P) that are extensively used to dissect P dynamics in the soil/plant system (Di et al., 1997; Frossard et al., 2011). However, these isotopes have a short half-life, 2 weeks for ^{32}P and 3 weeks for ^{33}P , and the use of radioactive isotopes in natural systems is restricted due to safety and environmental limitations. Another possibility is to take advantage of the stable isotopes of oxygen (^{16}O , ^{17}O , and ^{18}O) that are associated with P in phosphate.

Since the relative abundance of oxygen isotopes in phosphate varies naturally, accurate measurements of the oxygen isotope ratios in phosphate, $\delta^{18}\text{O}_\text{P}$ (and in the future maybe also of $\delta^{17}\text{O}_\text{P}$) may provide a unique mean of tracking phosphorus and its transformation in soils. This is possible because at the temperatures prevailing at the Earth surface, $\delta^{18}\text{O}_\text{P}$ mainly varies as a result of enzyme mediated reactions (Blake et al., 2001). While in the past, applications of $\delta^{18}\text{O}_\text{P}$ have been limited primarily to paleo-temperature studies utilizing biogenic apatite in teeth, bones, and shells (Longinelli and Nuti, 1973; Shemesh et al., 1983, 1988; Luz et al., 1984; Kolodny and Luz, 1988; Lecuyer et al., 1996), it has been lately suggested that $\delta^{18}\text{O}_\text{P}$ is a useful tracer of biogeochemical cycling of P in aquatic systems (Blake et al., 1997, 1998, 2005; Paytan et al., 2002; Colman et al., 2005; McLaughlin et al., 2006). The use of the natural abundance of oxygen stable isotopes associated to phosphorus, in order to trace the P cycle in terrestrial ecosystems, is still in its infancy with just a handful of published papers (Ayliffe et al., 1992; Mizota et al., 1992; Young et al., 2009; Zohar et al., 2010b), and analytical methods for purifying phosphate from soils are still under development (Tamburini et al., 2010; Zohar et al., 2010a; Weiner et al., 2011). While the impact of different P sources and soil age has been addressed in former studies, seasonal variations of different P fractions have not yet studied under field conditions.

Here we studied the seasonal variability of both the $\delta^{18}\text{O}_\text{P}$ in the plant available fraction P pool (i.e. extractable with an anion-exchange membrane), and in the HCl-extractable soil inorganic phosphate. This study was conducted in P-limited soils under natural vegetation. As fluctuations in soil humidity are known to strongly affect P transformations (Blackwell et al., 2010), we chose to study the seasonal variability in $\delta^{18}\text{O}_\text{P}$ across experimentally-manipulated rainfall gradients. In addition, we propose a framework for interpreting the variation of $\delta^{18}\text{O}_\text{P}$ in the soil/plant system.

2. METHODS

2.1. Study sites

Soil-samples were collected from a site in Israel with natural vegetation which previous studies describe in detail (Oren and Steinberger, 2008; Talmon et al., 2010). This site, located at $31^\circ23'41''\text{N}$, $34^\circ54'11''\text{E}$, has semi-arid climate with a mean annual precipitation (MAP) of

298 mm, while the average annual pan-evaporation for this area is 1942 mm. The soil is shallow (~10–20 cm depth) with large amounts of calcareous gravel and rock outcrops, and classified as Torriorthent (USDA) or Lithosol (FAO) (Singer, 2007). The total organic carbon content in the soil is $0.01 \text{ g(g soil)}^{-1}$, the pH is 7.8, bulk density is 1.35 g cm^{-3} , and the relative fractions of clay, silt, and sand are 26%, 42%, and 32%, respectively (Oren and Steinberger, 2008). The site rests on a calcareous Cenomanian–Turonian hard limestone bedrock. It is positioned on south-facing slopes and was fenced to exclude large grazers. Climatic data, including temperature, rainfall, 5 cm-depth soil temperature and moisture (determined by TDR, ThetaProbe™ by Delta-T Devices) are continually collected at the site.

Rainfall manipulation experiments took place at the site since 2002 (Sternberg et al., in press). In the drought plots, rain shelters, consisting of horizontal transparent plastic strips, decreased the amount of rainfall reaching the ground by ~30% without affecting the rain frequency. In the irrigation plots, water that was distributed through sprinklers concurrently with rainfall increased the water input to the soil by ~30%. The data of soil moisture and soil temperature from the three sensors per treatment, which were positioned under shrubs, was averaged. The water supply for irrigation comes from the National Water Carrier, which is fed by both groundwater and water carried from Lake Kinneret. Total area of the site is ~0.5 ha, with five replicates per treatment, and five control plots, arranged in blocks. Each plot is 25 m by 10 m.

We have sampled soil from this site four times during 2008: January 21st, April 9th, August 7th, and October 29th. The January sampling took place ~1 month after the first major increase in soil moisture in that growing season, and at the time of the largest difference in soil-moisture between treatments (Fig. 1). The soil moisture at the top 5 cm dropped below 10% in March – 1 month before the April sampling, and 5 months before the August sampling. The October sampling was done a few days after a major increase in soil moisture in the following wet season. Two samples per plot were taken from the entire soil depth and pooled together. Soil samples were always taken at the down-slope side of *Sarcopoterium spinosum* (Thorny burnet) dwarf-shrubs, which are the dominating vegetation type at the site, in order to focus on areas in which high biological activity takes place. The January samples for control were taken outside of the fenced area and must be treated with caution. Soil samples were dried at 40 °C, ground and sieved to 2 mm. To prevent biological activity from residual water that cannot be removed at 40 °C, the samples were stored at 4 °C until analysis.

2.2. Analytical methods

The available phosphate concentration in the soil samples was determined by extracting inorganic phosphate with sodium bicarbonate (Olsen et al., 1954) and by the anion-exchange resins used to measure the isotopic values (see below). Concentrations of the extracted phosphate were determined colorimetrically with the method of Murphy

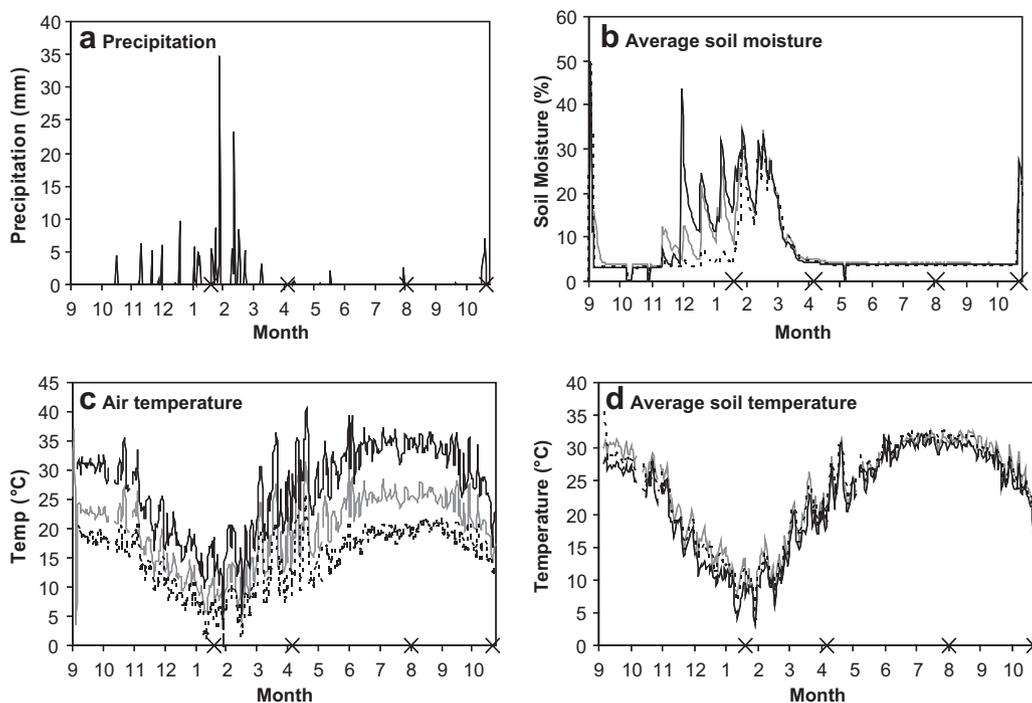


Fig. 1. Climate data for the site from September 2007 to November 2008. Soil moisture and temperature were averaged over three sensors per treatments. Soil sampling times are marked with “X” on the x-axis. In (b and d), gray, solid, and dashed lines represent control, irrigation, and drought treatments, respectively. In (c), they represent the daily average, maximum, and minimum, temperatures, respectively.

and Riley (1962) in duplicates, with an average difference between duplicates of 1.1%. The total element content of soils was measured at the Geological Survey of Israel, by fusing the soil samples with lithium metaborate followed by ICP analysis (Verbeek et al., 1982).

The $\delta^{18}\text{O}_\text{P}$ of the resin-extractable phosphate was measured using a method recently developed at the Hebrew University lab (Weiner et al., 2011). This method is based on combining established methods for soil phosphate extraction (Sibbesen, 1983) with a method for determining $\delta^{18}\text{O}_\text{P}$ in seawater (McLaughlin et al., 2004). Briefly, the available phosphate was first extracted from the soil by shaking it with anion-exchange membranes (BDH-55164) saturated with HCO_3^- . The phosphate was eluted from the membranes by shaking in 0.2 M HNO_3 overnight. SOM was removed from the extract by shaking it overnight with 20 ml of Superlite™ DAX-8 resin (Supelco/Sigma-Aldrich 20278). The phosphate was then extracted and purified by the precipitation of cerium-phosphate as described by McLaughlin et al. (2004). The precipitate was dissolved in nitric acid, the cerium was removed by a cation-exchange resin, and finally the phosphate was precipitated as silver phosphate.

The $\delta^{18}\text{O}_\text{P}$ of the HCl-extractable phosphate (which represents a large fraction of the total inorganic phosphate) was assessed by a method recently developed at the ETH lab (Tamburini et al., 2010), following the methods of Shemesh et al. (1983) and Tudge (1960) with some necessary adaptations for soils. This method is based on extraction with 1 M HCl, and purification through successive precipitations of ammonium phospho-molybdate and magnesium ammonium phosphate. Extracting this soil with ^{18}O

labeled water showed negligible incorporation of water oxygen during the process (Tamburini et al., 2010).

For isotopic composition determinations, five replicates of 0.3 mg silver phosphate were packed in silver capsules and introduced into a high temperature pyrolysis unit (HT-EA), where they were converted to CO in the presence of glassy carbon (Vennemann et al., 2002; Lecuyer et al., 2007). The HT-EA is interfaced in continuous flow (CF) mode, through a GC column, to an isotope ratio mass spectrometer (IRMS, Sercon 20-20). All isotopic values are given in the delta-notation vs. VSMOW. The average standard deviation between replicates was 0.3‰.

Due to the time consuming nature of the isotopic procedures outlined above, we have analyzed three (chosen in random out of five) soil samples replicates of each treatment for the resin extractable $\delta^{18}\text{O}_\text{P}$. For quality control, we randomly chose some soil samples and replicated the entire analysis. Since the HCl-extractable mineral P pool was not expected to show much seasonal variability, we have analyzed two samples per date for their $\delta^{18}\text{O}_\text{P}$, and only from the control plots.

All the isotopic measurements of the anionic membrane extractable P, and of the HCl-extractable mineral $\delta^{18}\text{O}_\text{P}$ were done at the Hebrew University of Jerusalem (HUJI). For comparison, one soil sample was analyzed in the Group of Plant Nutrition at ETH Zurich for the HCl-extractable mineral $\delta^{18}\text{O}_\text{P}$.

3. RESULTS

The soil total element contents is given in Table 1. The soil is dominated by silicate, and aluminum and iron oxides

Table 1
The soil total element content, in $\text{g}(\text{g soil})^{-1}$. (L.O.I. is Lost On Ignition), and the HCl-extractable PO_4 content.

SiO_2	0.5250
Al_2O_3	0.0865
Fe_2O_3	0.0440
TiO_2	0.0105
CaO	0.1240
MgO	0.0187
MnO	0.0008
Na_2O	0.0058
K_2O	0.0130
L.O.I.	0.1570
HCl-extractable PO_4	0.00034
Total	0.9855

which together contribute $0.66 \text{ g}(\text{g soil})^{-1}$. CaO contributes only $0.12 \text{ g}(\text{g soil})^{-1}$, despite the calcareous rock substrate. The rainfall in the 2007–2008 hydrological year was 191 mm (64% of MAP). The beginning of the hydrological year was unusually dry. In total, only 34 mm of rain fell in October, November, and December (Fig. 1a). This year was also the second year in a row of lower than average rainfall.

Volumetric soil moisture content at 5 cm depth ranged from 10% to 35% in the wet season (December–April), and from 3% to 5% in the dry season (Fig. 1b). In the early wet season, the irrigation and drought treatments had the strongest effect on soil moisture relative to the control (December 2007 to February 2008, Fig. 1b). Only small differences between treatments were observed later in the season, and in the beginning of the following hydrological year in October 2008.

Mean daily air-temperature ranged from 12°C in the wet season to 22°C in the dry season, and the daily temperature range was maximal in mid summer and minimal in mid winter (Fig. 1c). Soil temperature ranged from 9 to 31°C (Fig. 1d) with no considerable effect of the drought and irrigation treatments.

The Olsen-P contents are summarized in Fig. 2. Average content ranged from 7.7 to $11.2 \mu\text{g P g}^{-1}$, with a mean standard deviation of $1.9 \mu\text{g P g}^{-1}$ over five replicates of the same treatment and date. The Olsen-P in the irrigation plots was highest in January and dropped to significantly lower values (t -test $p < 0.05$) in April. In the drought plots, the drop in the same time frame is not statistically

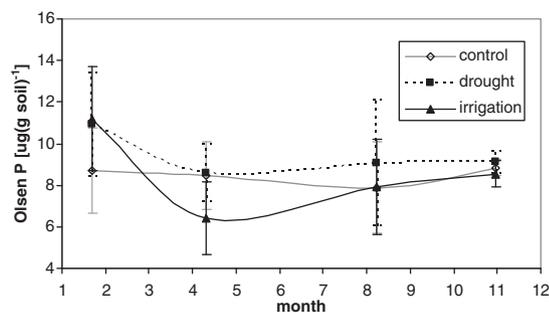


Fig. 2. Seasonal variability in the Olsen-P available phosphate in 2008, for the three treatments.

significant. For all treatments, no significant change was found in the concentrations from April onward.

The variations in resin extractable P contents are shown in Fig. 3. Average concentration ranged from 1.8 to $6.4 \mu\text{g P g}^{-1}$ with a mean standard deviation of $1.0 \mu\text{g P g}^{-1}$ over three replicates. The same main features described above for the Olsen-P were also observed for the resin extractable P, with two main differences: (1) the increase in October concentrations is much more pronounced; (2) the ratio between the observed variations and standard deviation was higher, so the decrease in the available-P from January to April is significant for both irrigation and drought plots ($p < 0.05$), as well as the lower April concentration in the irrigation plots relatively to the drought ones.

The content of the HCl-extractable P (measured in August) was $340 \mu\text{g P g}^{-1}$. The $\delta^{18}\text{O}_\text{P}$ values of this fraction measured at different points in time had an average value of 16.5‰ , with a standard deviation of 0.3‰ , which is the same as the HT-EA analytical uncertainty (Table 2). In contrast, the variability of the resin extractable P $\delta^{18}\text{O}_\text{P}$ was much larger (Fig. 4). Repeated analysis of replicates of the same soil samples gave usually a range similar to the HT-EA analytical uncertainty (Table 3), which indicates that the variability between samples is probably real. The highest variability, within and between treatments, was observed in January (average standard deviation of 1.2‰), and a lower variability close to the HT-EA analytical uncertainty of 0.3‰ , 0.5‰ , and 0.4‰ was observed in April, August, and October, respectively. The difference between

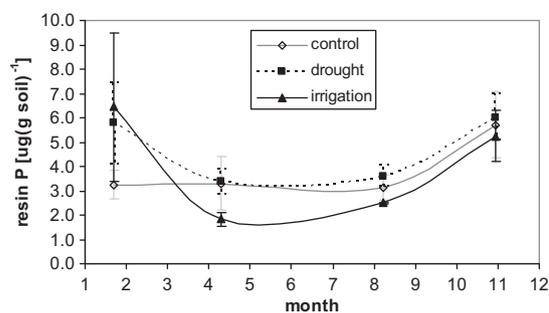


Fig. 3. Seasonal variability in 2008 for the resin-extractable available phosphate, for the three treatments.

Table 2
The $\delta^{18}\text{O}_\text{P}$ values (in permil vs. VSMOW) of HCl-extractable phosphate of samples from control plots. Similar values were found for samples taken from January to August, and in both labs.

Sample #	Lab	Month	$\delta^{18}\text{O}_\text{P}$
33	HUJI	1	16.4
60	HUJI	4	16.2
68	HUJI	4	16.1
125	HUJI	10	16.9
131	HUJI	10	16.5
104	ETH	8	16.7
Average			16.5
SD			0.3

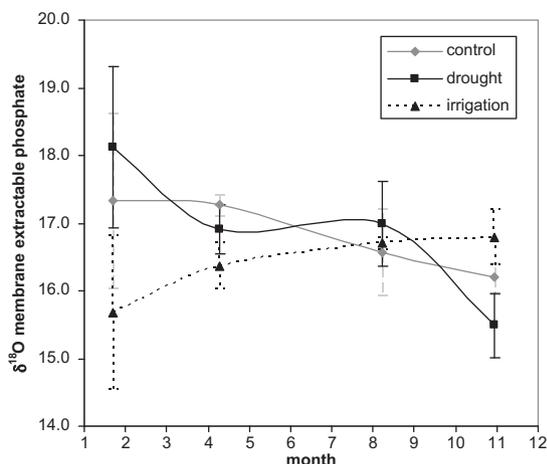


Fig. 4. Seasonal variations in 2008 of the $\delta^{18}\text{O}_\text{P}$ of available (resin extractable) inorganic soil phosphate, averaged over replicates from the same treatment and date. The error bars represent standard deviations.

treatments was also the largest in January. To determine if the high variability in January is due to an outlier of a given soil sample, we enlarged the number of replicates of drought and irrigation treatments to 5. These checks indicated that the variability observed was not an artifact (Table 3). The irrigation plots were found to be significantly ($p < 0.01$) lighter than drought plots with $\delta^{18}\text{O}_\text{P}$ values lower by 2.8‰. Smaller differences between treatments were found in the other seasons. However, the 1.3‰ heavier values of irrigation plots in comparison to drought plots in October were also found to be significant ($p < 0.05$). For all months, the modest number of replicates per treatment limits the strength of the statistical tests. No correlation was found between the concentration of resin extractable P and its $\delta^{18}\text{O}_\text{P}$ ($R^2 = 0.005$).

4. DISCUSSION

4.1. Phosphate concentrations

The contents of the soil Olsen and resin P were of similar magnitude. These methods are based on different principles and do not extract the same forms of P (Frossard et al., 2000). The type of resin used in this work acts as a sink that is sorbing inorganic P, leading to a decrease in P concentration in the soil solution and to continuous desorption of inorganic P from the solid phase of the soil. The forms of inorganic P that are extractable by such resins have also been shown to be isotopically exchangeable within the time of resin extraction by Schneider and Morel (2000). We can therefore state that the resin extracts a fraction of available P, but most probably not all of it. Beside inorganic P, these resins extract also limited amounts of organic P (Rubæk and Sibbesen, 1993) and soil organic matter. The small amount of organic matter extracted by this method makes it interesting for the measurement of $\delta^{18}\text{O}_\text{P}$ as the presence of organic matter can strongly bias this analysis and requires special treatment (Tamburini et al., 2010; Weiner et al., 2011). Unfortunately, the resin P method is not used

Table 3

The $\delta^{18}\text{O}_\text{P}$ values (vs. VSMOW) of the available resin-extractable inorganic phosphate, and the available phosphate concentrations in all three treatments. Samples were taken in January, April, August, and October 2008. Some samples were analyzed more than once, for quality control.

Sample #	Month	Treatment	$\delta^{18}\text{O}$	P [$\mu\text{g}(\text{g soil})^{-1}$]
20	1	Control	16.4	3.66
33	1	Control	18.2	2.85
21	1	Drought	19.1	6.33
22	1	Drought	18.5	6.68
23	1	Drought	16.7	4.38
23	1	Drought	16.2	3.80
25	1	Drought	18.4	7.48
26	1	Drought	18.3	3.11
24	1	Irrigation	16.9	9.81
24	1	Irrigation	16.0	7.19
28	1	Irrigation	17.7	8.68
29	1	Irrigation	14.9	4.05
29	1	Irrigation	15.2	4.54
32	1	Irrigation	15.9	10.80
34	1	Irrigation	14.5	3.32
34	1	Irrigation	14.6	3.12
56	4	Control	17.4	1.76
68	4	Control	17.4	4.37
68	4	Control	17.0	3.69
69	4	Control	17.3	3.40
59	4	Drought	17.1	2.80
63	4	Drought	16.5	3.62
72	4	Drought	17.1	3.76
55	4	Irrigation	16.7	1.50
58	4	Irrigation	16.1	1.93
71	4	Irrigation	16.2	2.04
91	8	Control	17.2	2.86
103	8	Control	16.7	3.73
104	8	Control	15.9	2.82
95	8	Drought	16.4	4.10
97	8	Drought	17.6	3.24
100	8	Drought	17.0	3.49
93	8	Irrigation	16.8	2.47
105	8	Irrigation	16.6	2.52
124	10	Control	16.0	5.70
125	10	Control	16.1	4.36
131	10	Control	16.5	7.07
122	10	Drought	15.0	5.00
127	10	Drought	15.5	6.13
136	10	Drought	16.0	6.88
123	10	Irrigation	16.3	3.88
134	10	Irrigation	16.7	5.14
134	10	Irrigation	17.1	5.61
134	10	Irrigation	17.1	6.35

often in ecosystem studies, making comparisons between studies difficult.

The Olsen method, on the other hand, has been widely used to assess P availability (Frossard et al., 2000) including in semi-arid shrublands similar to the one studied here (Henkin et al., 1998; Sardans et al., 2008a). In this method, the soil is extracted with NaHCO_3 0.5 M at pH 8.5. As a result, inorganic and organic P are solubilized. Olsen and Sommers (1982) argued that in calcareous soils Ca bounds to inorganic P can be precipitated by the added CO_3^{2-} as CaCO_3 , leading to the release of inorganic P to the solution,

while in neutral or slightly acidic soils, sorbed inorganic P is released from iron and aluminum oxides as the pH of the soil solution increases. More recently [Rahnemaie et al. \(2007\)](#) demonstrated that an increased CO_3^{2-} concentration led to inorganic P desorption from the surface of a goethite. However, given the fact that forms of P are very often ill defined in soils, it is difficult or impossible to ascribe a specific mechanism to a given extraction. Any type of mild extractant will solubilize a mixture of P forms. Hence, it is better to say that the Olsen extraction solubilizes forms of inorganic P that are loosely sorbed on soil minerals. It is important to note that although the proportion of available inorganic P extracted by the Olsen method is always high, which explains its success to assess soil available P for plant in routine analyses, its exact proportion is variable and not predictable. For example, [Demaria et al. \(2005\)](#) estimated that the fraction of non-exchangeable (considered as unavailable) inorganic P extracted by the Olsen method ranged in four soils from 8% to 24% of the inorganic P. This unavailable phosphate can potentially bias isotopic measurements of the Olsen-P extract. The Olsen method extracts also a fraction of soil organic P. The lability of this organic P fraction is considered to be high, but can in some cases be limited ([Buehler et al., 2002](#); [Chen et al., 2002](#)). The relatively high amounts of organic matter extracted by the Olsen method render a precise measurement of the $\delta^{18}\text{O}$ -P in inorganic P difficult, as the presence of organic matter can strongly bias this analysis and requires special treatment ([Tamburini et al., 2010](#); [Weiner et al., 2011](#)). Nevertheless, a method to measure $\delta^{18}\text{O}$ -P in soil bicarbonate extracts was recently suggested ([Zohar et al., 2010a](#)).

A comparison of the variations in the Olsen and resin extracts shows similar absolute magnitude. The resin-extractable fraction is contained in the Olsen fraction, indicating that most of the variations are in the resin-extractable fraction. The low contents of Olsen P found in our study are comparable to those found in another site in Israel by [Henkin et al. \(1998\)](#) on their unfertilized plots. They reported that the contents strongly limited the growth of the shrub *S. spinosum*, which is also dominating at our site. Response to P fertilization was also found in semi-arid grassland soils in the area of the research site ([Grunzweig and Korner, 2003](#)). We can conclude from this comparison that P was limiting plant growth at our site.

The available-P concentrations (both the Olsen-P and resin-extractable) show similar seasonal variability, with a maximum in January, a drop to lower values in April, and no change during the dry summer months. The increase in available P content after the first rains in October is seen much more clearly with the resin extraction than with the Olsen extraction. To the best of our knowledge, seasonal variations in Mediterranean soils were only reported once before ([Sardans et al., 2008a](#)). These authors showed that the Olsen inorganic P at both the 0–15 and 15–30 cm horizons decreased from autumn to winter. Our control plots do not show the January maximum, probably because at that date the control samples were taken outside of the fenced area. This result seems to indicate that the grazing pressure removed available-P from the soil, and warrants more research in the future. In the irrigation plots, the

resin-extractable available-P dropped in 72% from January to April, while in the drought plots, a smaller decrease of only 41% was evident.

The seasonal variations we observed in the available phosphate are suggested here to result from biological processes. The main biological processes affecting soil inorganic P are: (1) mineralization of organic P, which releases inorganic P to the soil; (2) plant and microbial uptake of P, which removes inorganic P. Both processes rates are expected to increase in spring, because of the increase in temperature and the presence of moisture. The lower available inorganic-P at the end of spring seems to indicate that the uptake rate was higher than the rate of P release prior to the sampling. The higher available P contents observed in fall and winter might be linked to two different phenomena. In October, the first rains might have provoked an osmotic shock on soil microbes which resulted in a large pulse of available P to the solution. It is indeed known that drying and rewetting cycles can lead to strong pulses of available P ([Blackwell et al., 2010](#)). The lower temperatures in January might then have hindered P uptake by plants and microorganisms explaining the accumulation of soil available P. The lower values in the irrigation plots, observed in spring and summer, are a possible indication of faster microbiota and plant uptake in wetter plots, compared to drought plots. Further research that will test the P concentration in the soil microbiota and the various plant organs in this site is needed for confirming this prediction. There is one study ([Sardans et al., 2008b](#)) that did find a 21% decrease in *Globularia alypum* leaves P concentration at drought treatment plots, but another study ([Menge and Field, 2007](#)) did not find an effect in green *Avena barbata* leaves from an irrigation treatment. The $\delta^{18}\text{O}_\text{P}$ values can possibly also shed light on such questions.

4.2. Oxygen isotopes variations

The results above demonstrate significant seasonal, and between treatments, variations in the available-P oxygen isotopic composition. This is the first time that such variations are shown under field conditions. These variations do not simply correlate with the changes in available-P, indicating that the oxygen isotopes carry additional information. In order to determine which process controls the isotopic composition of oxygen in available and HCl-extractable phosphate, as well as the seasonal changes in this composition, we shall first discuss the possible isotopic effect in the plant–soil system.

4.2.1. Expected oxygen isotopic effects in the terrestrial phosphate cycle

The isotopic composition of phosphate in soils is likely to be controlled by different processes ([Fig. 5](#), see also [Zohar et al., 2010b](#)). First, primary phosphate is supplied to soils by dissolution from minerals, mainly apatite, supplied by the bedrock and airborne dust. These sources have a range of $\delta^{18}\text{O}_\text{P}$ values of $\sim 5\text{‰}$ for igneous rocks (e.g. [Taylor and Forester \(1979\)](#)) and 11–35‰ for marine phosphorites ([Shemesh et al., 1983](#)). The expected value for rock and dust in the study area is about $\sim 20\text{‰}$ ([Shemesh et al.,](#)

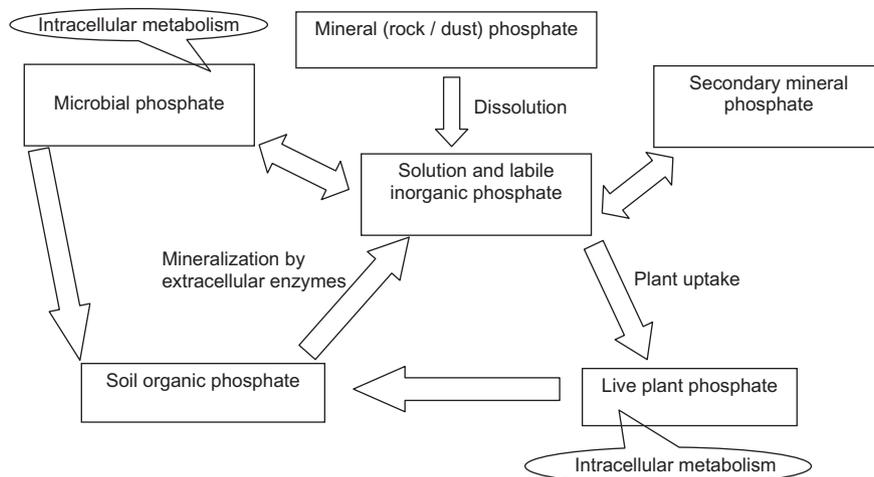


Fig. 5. Suggested conceptual scheme for the main pools, fluxes and isotopic effects in the soil–plant phosphorus cycle.

1983). There is no fractionation in dissolution of apatite (Lecuyer et al., 1999).

In the second step, phosphate is assimilated into plants or the soil microbial community. Fractionations, if any, involved in this step are unknown. A small fractionation ($\epsilon = -3\text{‰}$) was observed for *Escherichia coli* under laboratory conditions (Blake et al., 2005). However, this effect may not represent the soil microbial population under natural conditions and can be very different from that of fungi and plants. In the third step, we expect that intra-cellular enzymatic activity within plants will result in isotopic equilibrium between oxygen in phosphate and oxygen in water, as was found for other organisms. The known equilibrium fractionation for this reaction is temperature dependent (Longinelli and Nuti, 1973; Blake et al., 2005)

$$\delta^{18}\text{O}_P = \delta^{18}\text{O}_{\text{water}} + (111.4 - T)/4.3 \quad (1)$$

(where T is the temperature in $^{\circ}\text{C}$)

This equilibrium fractionation stems from a reaction catalyzed by the intra-cellular enzyme pyrophosphatase (Blake et al., 2005). This equilibration will take place in the microbial biomass and in the plant's stems and roots, with water with the same $\delta^{18}\text{O}$ as the soil water, and in the leaves, with water that are isotopically enriched (White, 1988).

Fractionation and isotopic exchange are expected also to occur in the fourth step: organic matter mineralization. The biota releases organic P into the soil, where phosphate-scavenging enzymes break down large and complex organic molecules and release inorganic phosphate, which becomes available for biological uptake (Blake et al., 2005; Liang and Blake, 2006). For example, when phosphate is released from phosphomonoesters, which are the most common organic-P compounds in soils (Turner et al., 2002), by the extracellular enzyme alkaline-phosphatase, one of the oxygen atoms is acquired from water, with a kinetic fractionation of $-30(\pm 8)\text{‰}$ (Liang and Blake, 2006). The other three oxygen atoms in the inorganic phosphate are then inherited from the organic phosphate that alkaline-phosphatase hydrolyzed broke down. Similar

reaction is catalyzed by 5'-nucleotidase, but with a fractionation of $-10(\pm 1)\text{‰}$. When the substrate is phosphodiester, two C–O–P bonds must be broken, and two oxygen atoms are incorporated with associated isotopic fractionation, into the released phosphate molecule. The fractionation associated with diesters hydrolysis was reported to be substrate related (Liang and Blake, 2009). The isotopic exchange and fractionations associated with other common phosphatases in soils like acid-phosphatase, or phytase are currently unknown.

Phosphate released from organic-P re-enters the inorganic pool, and then can be either assimilated by plants or by the soil microbial population, sorbed to soil, or precipitate and form secondary phosphate minerals. Laboratory experiments have shown that precipitation of apatite involves only very small isotopic fractionations of $\sim 1\text{‰}$ (Liang and Blake, 2007). A recent study (Jaisi et al., 2010) has found negligible fractionation between phosphate sorbed into iron oxides and the one in aqueous-phase at equilibrium, and only small fractionations ($\sim 2\text{‰}$) in the early stages of phosphate sorption. Other sorbing reactions in soils might have different isotopic effect associated with it. Phosphate assimilated into microbial biomass is expected to reach isotopic equilibration with intra-cellular water that has $\delta^{18}\text{O}$ similar to that of soil water. This phosphate is then released to the solution upon microbial cells death caused by starvation, or strong changes in environmental conditions such as drying and rewetting cycles (Blackwell et al., 2010; Oberson et al., 2011). Phosphate can then be released in organic and/or inorganic forms depending on the forms of P originally present in the microorganisms (Bünemann et al., 2011). In addition, bacteria excrete both organic and inorganic phosphate (Currie and Kalf, 1984; Jansson, 1988). This is in contrast to Zohar et al. (2010b) who assumed all the phosphate released from the bacteria is inorganic. Of course, all the different steps and processes detailed above can operate at the same time in varying rates.

4.2.2. Interpreting the measured $\delta^{18}\text{O}_P$ Values

In order to interpret the measured values, we should first consider the expected equilibrium values based on the

temperatures and the expected isotopic composition of soil water in our site. We will assume that equilibration occurs within the time scale of a few weeks, as was found in a recent soil incubation experiment (Zohar et al., 2010b). The soil temperature at the site is around 10 °C in January, 17 °C in April, and 21 °C in October. A higher temperature of ~30 °C is observed in August, but is considered here to be irrelevant, since it occurs when the soil is dry and biological activity is minimal. The accumulated precipitation $\delta^{18}\text{O}$ varies between ~-4‰ in October and January to -6‰ in April (Ayalon et al., 1998). Based on this data we can estimate that the $\delta^{18}\text{O}_\text{P}$ in equilibrium will be around 19.5‰ in January, 16‰ in April, and 17‰ in October. However, evaporation at the top part of the soil can cause ^{18}O enrichment of the soil water. In addition, the irrigation water supply was from the water grid which sometimes switches between the use of groundwater with the same isotopic composition as average rainwater (-5‰), to Lake Kinneret water with isotopic composition of about 0‰. Thus, these two last effects can cause higher equilibrium values than those we have calculated above.

One should also consider that equilibration in plants leaves is expected to create plant litter, with higher $\delta^{18}\text{O}_\text{P}$ values than those resulting from equilibration in the soil. That is because leaf water is isotopically enriched, with typical values for the study area of about -5‰ for soil water (Ayalon et al., 1998) compared to measured values in the range of +5‰ to above 20‰ for leaf water in Israel (Landais et al., 2006). Thus, for the same temperature, the $\delta^{18}\text{O}_\text{P}$ in leaf litter is expected to be 10–25‰ heavier.

The calculated equilibrium values can be now compared to the measured values. The average HCl-extractable phosphate $\delta^{18}\text{O}$ was 16.5‰ at this site. This value is lower than the typical values of ~20‰ (Shemesh et al., 1983) for calcareous rocks in this region. These rocks are probably the main source of phosphate to the soil, either from the rocky substrate, or from wind-blown dust. However, the HCl-extract $\delta^{18}\text{O}_\text{P}$ values fall in the range calculated for equilibration with soil water (16–19.5‰). Thus, these results seem to indicate that most of the inorganic phosphate in this site has been recycled through the soil microorganisms at some point of the soil history. HCl-extractable and total inorganic $\delta^{18}\text{O}_\text{P}$ values which are close to equilibrium or slightly higher were also found in a Mediterranean soil in Israel, in sites in Europe (Tamburini et al., 2010), and in other soil studies (Ayliffe et al., 1992; Mizota et al., 1992), excluding extremely young soils which still retain the source material signature. Values reaching equilibrium were also observed in lab incubation experiments (Blake et al., 1997; Zohar et al., 2010b). However, explaining how the soil phosphate got equilibrated with the soil water is not as straightforward as it may seem.

Equilibration of phosphate with soil water is expected to occur within the microbial community, and in roots. Roots and microorganisms release some of this phosphate in the inorganic form and some in organic forms. As discussed above, mineralization of organic-P is associated with kinetic effects (with fractionation of up to -38‰), that lead to values of inorganic $\delta^{18}\text{O}_\text{P}$ which are lower than the equilibrium value. For example, if we assume activity of

alkaline-phosphatase with a fractionation of -30‰, a water $\delta^{18}\text{O}$ of -5‰, and a phosphomonoester substrate, the value for the released $\delta^{18}\text{O}_\text{P}$ is 8.75‰ lighter than the organic-P source. Assuming that the organic-P is in equilibrium with the soil water as described above, leads to a $\delta^{18}\text{O}$ of released phosphate of ~8‰ (16.5–8.75‰), which is far from the observed values (16.5‰). Similarly, other extracellular hydrolysis reactions are also expected to produce inorganic P with lower values than those of equilibrium (Liang and Blake, 2009).

The values we observed of resin extractable-P close to equilibrium could be then explained in three ways: (1) if the main P flux in the soil is through excretion of inorganic-P; (2) if some mechanisms, such as extracellular pyrophosphatase activity, cause equilibration of the inorganic-P released from organic-P. Such a mechanism was proposed for explaining observations in the ocean (Colman et al., 2005), and indeed, free pyrophosphatase activity is observed in soils (Baligar et al., 1991; Dick and Tabatabai, 1983); (3) if values similar to the ones expected from equilibrium were accidentally produced by the contrasting effects of lighter oxygen originating from mineralization of microbial and roots organic-P, the heavier oxygen originating from leaf litter and primary mineral dissolution, and the possible enrichment effect of uptake. Although we do not consider the last explanation as very likely, more research is needed to determine which of these three explanations is correct.

While the HCl-extractable phosphate $\delta^{18}\text{O}$ values were constant throughout the year, we did observe seasonal changes in the available $\delta^{18}\text{O}_\text{P}$. Having variations in the available fraction, with no measurable changes in the HCl extract which contains also the available fraction, is possible since the HCl extract is two orders of magnitude larger. In addition, larger seasonal changes in the available P are expected, because this labile fraction is much more dynamic. It is interesting however to note, that the average isotopic values of the available phosphate over all treatments in April and in August were 16.8‰ (SD = 0.3‰), and 16.7‰ (SD = 0.7‰) for the entire year. These values are indistinguishable from the average value for the HCl-extractable phosphate (16.5‰). This result seems to indicate that isotopic value of the HCl-extractable phosphate integrates and records the values of available-phosphate.

The variations between treatments and within treatments in the values of available $\delta^{18}\text{O}_\text{P}$ were larger in January and October (Fig. 4). The January samples were taken about one month after the first major increase in soil moisture in that growing season, and the October sampling was done a few days after the same event in the following season (Fig. 1). Hence, we infer that the increase in soil moisture is responsible for these observed isotopic effects. Soil temperatures at the drought and irrigation plots were practically identical (Fig. 1d), thus temperature effects did not play an important role. Geochemical processes like dissolution and sorption are associated with isotopic effects which are small (see previous section) compared to the range of 4.6‰ between samples we have observed; hence we conclude that soil-moisture-controlled biological processes are the main drivers of the observed $\delta^{18}\text{O}$ variations. Similar

conclusion was drawn for a soil experiment in the lab (Zohar et al., 2010b).

The four main biological processes we must consider are equilibration within plants, uptake by plants and microbes, extracellular mineralization by phosphatases, and equilibration within the soil microbial community. As discussed above, there is limited understanding of the fractionation associated with extracellular mineralization and uptake. However, we can probably rule out plant uptake as affecting the fall and early winter. That is since plant growth at this site peaked up only later in the season in these years. This is indicated by satellite derived vegetation index for 2.25 km² around the site area (MODIS NDVI) (Oak Ridge National Laboratory Distributed Active Archive Center, 2009). This index shows that for both 2008 and 2009 the seasonal increase in the surface greenness started only in February. We thus turn to equilibration and mineralization as possible drivers of the variation we observed.

The highest $\delta^{18}\text{O}_\text{P}$ values observed in January are close to the expected value for equilibrium in this month, which is 19.5‰. As discussed above, mineralization of microbial and roots organic-P is expected to result in $\delta^{18}\text{O}_\text{P}$ which is lighter than the equilibrium values. Thus, such mineralization will first cause a decrease in $\delta^{18}\text{O}_\text{P}$, but later, as this phosphate is turned over by the microbial population or equilibrated by extracellular pyrophosphatase, its original signature will be erased and the $\delta^{18}\text{O}_\text{P}$ values will return to equilibrium values. This description fits quite nicely the differences between the drought and irrigation plots in January. The drought plots show mostly the equilibrium values, while in the irrigation plots, the wetter soil (Fig. 1) promoted faster mineralization. Note that the possible use of Lake Kinneret water for irrigation cannot be responsible for the results, since it would have driven the irrigation plots values in the opposite direction than observed. Different mineralization rates can also explain the larger variability between replicates of the same treatment in January. It is very reasonable that at different sampling spots the mineralization was slightly faster than in others, especially given the high spatial heterogeneity of this shallow soil which is dominated by rock outcrops. Also, different spots might have different contributions of leaf litter with heavier $\delta^{18}\text{O}_\text{P}$.

According to the satellite vegetation index, peak vegetation activity in 2008 was in March. The April sampling thus probably reflects the results of this period of maximum plant and soil activity, resulting from a combination of higher temperatures and wet soil. This is also indicated by the March–April peak in soil respiration at this site in 2006 and 2007 (Talmon et al., 2010). This high activity caused fast recycling, or fast extracellular equilibration, which erased any difference between treatments. The highest $\delta^{18}\text{O}_\text{P}$ values in April are lower than those in January, and are consistent with the temperature-controlled equilibrium.

The August values show no isotopic changes from April. This is a very reasonable result, because we do not expect any biological phosphate turnover in these extremely dry summer months, in which soil respiration rates are very low (Talmon et al., 2010). This result is also consistent with the lack of observed change in the available phosphate concentrations. In contrast, the October samples do show a decrease

in the $\delta^{18}\text{O}_\text{P}$ of the drought plots, which reaches lower values than that of the irrigation plots. This can be explained by lysis of cells caused by rewetting of the soil, which released organic-P that was later mineralized to inorganic-P with low $\delta^{18}\text{O}$ values. The wetter soil in the irrigation plots (and to a lesser extent in the control plots) prompted a change in the isotopic composition towards equilibrium values (about 17‰ in that month). Different relative contributions of leaf litter could have also played a role.

The results above provide a first picture of seasonal variability in available soil phosphate $\delta^{18}\text{O}_\text{P}$. As the discussion above shows, we are far from fully understanding the controls on this variability. Much more lab and field studies must be conducted before all the relevant mechanisms can be accounted for. However, the existence of such variability points to the potential use of oxygen isotope geochemistry to better understand P turnover in soils. For example, if our explanation above is proved to be true, then pulse-chase experiment in which the rate of change in the phosphate isotopic value is traced, can be used to estimate the rate of phosphorus turnover in the soil.

5. CONCLUSIONS

We report here the first measurements of available and HCl-extractable phosphate $\delta^{18}\text{O}$, from natural semiarid and low available P soils. The $\delta^{18}\text{O}_\text{P}$ values of the HCl-extract were close to the values expected for equilibrium with the soil water, at the average temperature of the growing season, and to the average values of the available $\delta^{18}\text{O}_\text{P}$. As a result we conclude that HCl fraction contains mostly phosphate that was turned over by biological activity.

In contrast to the isotopic values of HCl-extract, which were constant throughout the year, the available phosphate $\delta^{18}\text{O}_\text{P}$ values showed seasonal and between treatments variability. The highest variability was found in January and the lowest one in April. We suggest that the highest values measured in January and April represent equilibrium, while the lower ones bear signature of extracellular mineralization. The difference between and within treatments is explained by varying rates of biological activity. The different maximum values in January and April are consistent with temperature dependent equilibrium values.

The equilibrium values can result from either an inorganic-phosphate excretion flux which dominates the soil P-cycle, or from extracellular pyrophosphate activity. In contrast, the standard argument of “fast recycling” falls short of explaining the observed equilibrium values, if the organic-P flux from decomposing bacteria and roots is considerable.

Our results show that the $\delta^{18}\text{O}$ of soil phosphate can be used to trace P turnover in soils. However, much more laboratory and field studies must be conducted before all the relevant mechanisms acting in the soil/plant system can be accounted for.

ACKNOWLEDGMENTS

This research was partly supported by ISF Grant No. 870/08 and GIF Grant No. 2174–1762.8/2007. The authors thank T.

Venneman for providing Ag_3PO_4 standards, N. Teutsch and Y. Kolodny for valuable discussions, and Y. Sherer for technical support. The study site is part of the GLOWA Jordan River project (www.glowa-jordan-river.de) supported by the German Federal Ministry of Education and Research (BMBF) in collaboration with the Israeli Ministry of Science and Technology (MOST).

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Associate editor: Timothy W. Lyons