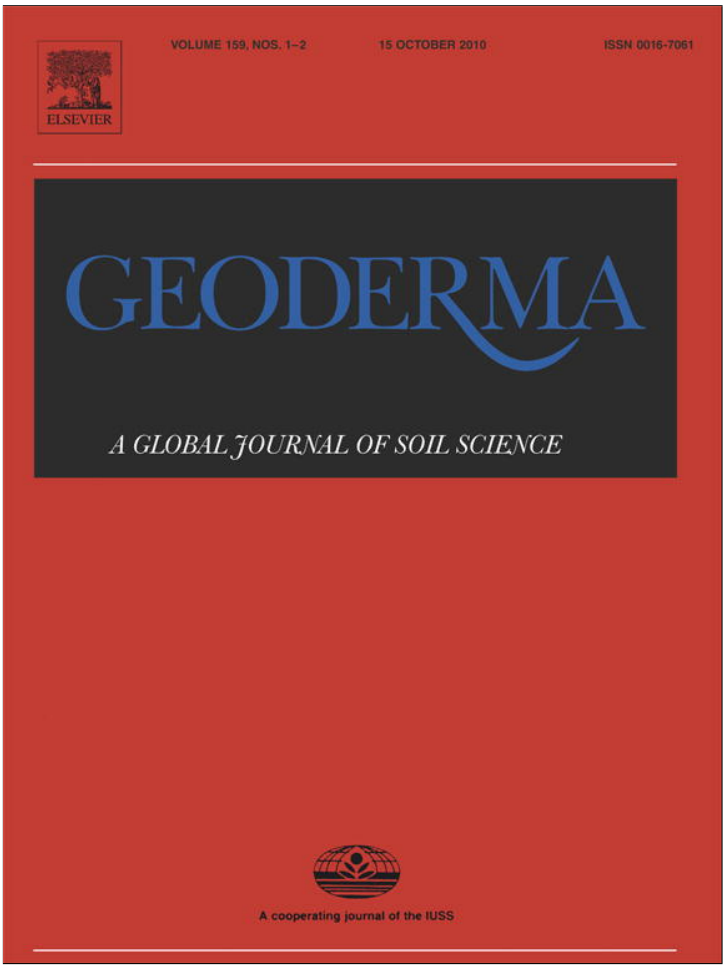

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Phosphorus dynamics in soils irrigated with reclaimed waste water or fresh water — A study using oxygen isotopic composition of phosphate

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ABSTRACT

Transformations of phosphate (Pi) in different soil fractions were tracked using the stable isotopic composition of oxygen in phosphate ($\delta^{18}O_p$) and Pi concentrations. Clay soil from Israel was treated with either reclaimed waste water (secondary, low grade) or with fresh water amended with a chemical fertilizer of a known isotopic signature. Changes of $\delta^{18}O_p$ and Pi within different soil fractions, during a month of incubation, elucidate biogeochemical processes in the soil, revealing the biological and the chemical transformation impacting the various P pools. P in the soil solution is affected primarily by enzymatic activity that yields isotopic equilibrium with the water molecules in the soil solution. The dissolved P interacts rapidly with the loosely bound P (extracted by bicarbonate). The oxides and mineral P fractions (extracted by NaOH and HCl, respectively), which are considered as relatively stable pools of P, also exhibited isotopic alterations in the first two weeks after P application, likely related to the activity of microbial populations associated with soil surfaces. Specifically, isotopic depletion which could result from organic P mineralization was followed by isotopic enrichment which could result from preferential biological uptake of depleted P from the mineralized pool. Similar transformations were observed in both soils although transformations related to biological activity were more pronounced in the soil treated with reclaimed waste water compared to the fertilizer treated soil.

1. Introduction

Phosphorus (P) is a required nutrient for all living organisms and low P availability could limit growth and productivity. Specifically, P limitation may impact agricultural yield (Khasawneh et al., 1980; Bakker et al., 2005). Therefore P is added to cultivated soils worldwide through chemical or organic (e.g., manure and sewage sludge) fertilization. Water scarcity in arid places (like Israel) has led to the use of reclaimed waste water (RWW) for irrigation. This RWW also serves as a source of nutrients, including P. However, fertilization with biosolids or RWW may be associated with accumulation of excess labile P in top soils (Sui et al., 1999; Hansen et al., 2004; Tarchitzky, 2004) and consequently could become an environmental threat to adjacent water bodies, causing ecological imbalance and eutrophication (Tunney et al., 1997).

The most bioavailable form of P is inorganic orthophosphate ($H_2PO_4^-/HPO_4^{2-}$ hereafter referred to as Pi), which is also the most abundant stable form of free dissolved inorganic P in neutral pH soil solution (Lindsay, 1979). While P has only one stable isotope (^{31}P), oxygen has three stable isotopes (^{16}O, ^{17}O, ^{18}O) which could be used as isotope tracers for tracking Pi sources and transformations. Under common surface temperature and pH conditions the P–O bond in PO_4^{3-} is relatively strong and resists inorganic hydrolization for long periods (Shemesh et al., 1983; Saaby Johansen et al., 1989). However, enzyme mediated biological activity could break the P–O bond in processes that involve isotopic fractionation (Longinelli et al., 1976; Blake et al., 1997; Paytan et al., 2002). Intracellular as well as extracellular enzymes are expressed by various organisms for the utilization and cycling of P and may play a role in determining the oxygen isotopic composition of Pi ($\delta^{18}O_p$, $^{18}O/^{16}O$ relative to VSMOW (Vienna standard mean ocean water) international reference standard). Different enzymatic processes induce different isotopic fractionation, allowing the $\delta^{18}O_p$ to elucidate such processes as long as their collective isotopic imprints do not cancel each other out (e.g., as long as one process controls the overall isotopic signature), (Blake et al., 2005).

The most dominant enzymatic process controlling $\delta^{18}O_p$ in the environment is the intracellular activity of pyrophosphatase (PPase) (Blake et al., 2005), which involves equilibrium isotopic exchange.

Abbreviations: P, phosphorus; Pi, inorganic orthophosphate; Po, organic phosphate; Pt, total P; RWW, reclaimed wastewater; FWF, fertilizer amended freshwater; RWW soil, soil treated with RWW; FWF soil, soil treated with FWF; $\delta^{18}O_p$, oxygen isotopic composition of Pi; PPase, pyrophosphatase; APase, alkaline phosphatase.

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The isotopic equilibrium of Pi has been described by Longinelli and Nuti (1973) in the following empirical equation:

$$T(^{\circ}C) = 111.4\text{–}4.3\left(\delta^{18}O_p - \delta^{18}O_w\right) \quad (1)$$

where $\delta^{18}O_w$ is the isotopic composition of oxygen in water and T is temperature in degrees Celsius. It follows from the equation that $\delta^{18}O_p$ is enriched relative to $\delta^{18}O_w$, however, the difference between them decreases as temperature increases. This equilibrium relation has been observed in tissues of a variety of organisms, including fish and mammals (Kolodny et al., 1983), bacteria and algae (Blake et al., 1997; Paytan et al., 2002; Blake et al., 2005) and used for reconstruction of paleoclimates (e.g., Ayliffe and Chivas, 1990; Fricke et al., 1998). Recently, $\delta^{18}O_p$ in aquatic systems has been used as a tracer of Pi sources as well as for deciphering the biological utilization and turnover of Pi in these systems (Longinelli, 1989; Colman et al., 2005; McLaughlin et al., 2006a,b; Elsbury et al., 2009). This is based on the assumption that extensive recycling and turnover will lead to isotopic equilibrium while deviation from equilibrium may reflect source signatures or other processes that do not result in isotopic equilibrium. Specifically, extracellular remineralization and hydrolization of organic P (Po) compounds to form Pi, by phosphohydrolase enzymes such as alkaline phosphatase (APase) and 5′-nucleotidase, involves incorporation of oxygen atoms from ambient water with an isotope fractionation of −10‰ to −30‰ (Liang and Blake, 2006). These enzymatic processes are expected to occur in soils through the activity of microorganisms and can impact dissolved Pi concentrations and $\delta^{18}O_p$ values. Uptake and utilization of Pi by plants or soil microorganisms is also associated with isotopic fractionation where Pi with light isotopes is preferentially utilized leaving the residual pool enriched (Blake et al., 2005).

Isotopic composition of soil Pi may also be affected by geochemical processes. Precipitation of apatite minerals is accompanied by a small oxygen isotope fractionation in the range of +0.7‰ to +2‰ (Zheng, 1996; Blake et al., 1997). Similarly, adsorption or precipitation with sesquioxides and hydroxides imprints a small positive isotope effect (Jaisi et al., 2010). These geochemical processes may alter Pi isotope ratio in the soil and can be described by isotopic mass balance models (e.g., Markel et al., 1994).

Labile P concentrations, defined as P in the soil solution and P which is loosely bound (Tiessen and Moir, 1993; Falkiner and Polglase, 1999; Guggenberger et al., 2000), are primarily controlled by the soil's various binding agents such as sesquioxides, clay and organic matter surfaces (via adsorption/desorption processes) and apatite minerals (by precipitation/dissolution), while highly recalcitrant soil P does not contribute to the labile pool (Tiessen and Moir, 1993). Understanding P transformations among the various distinct soil fractions may shed light on P availability. Utilization of $\delta^{18}O_p$ for tracking P transformations in soil has so far been limited to tracking P dissolved in the soil solution (Larsen et al., 1989; Middelboe and Saaby Johansen, 1998). This is primarily due to lack of a method that addresses the complexity of extracting and analyzing Pi from other fractions of soil for oxygen isotopes. Zohar et al. (submitted) described a method to produce silver phosphate from different soil extract solutions, removing the barrier for using isotope tracing for the study of P transformations in soil.

In this paper we applied $\delta^{18}O_p$ to study P transformations in two soil samples, over one month of incubation in the laboratory under controlled conditions. In this experiment, the soil samples were irrigated with either RWW or freshwater amended with fertilizer (FWF) and Pi transformations were tracked by determining changes in $\delta^{18}O_p$ of the various soils' Pi pools. Using this isotope tracing technique enabled elucidation of processes which could not have been tracked by conventional methods and thus significantly enhanced our understanding of the biogeochemical processes that control P fate in soil. Application of this isotope tracing technique may further elucidate soil P transformations, mobility and bioavailability in future research.

2. Materials and methods

2.1. Experimental design

Soils with different irrigation history (field irrigation with either RWW or FWF), were treated respectively with RWW or FWF containing similar Pi concentrations. RWW or FWF were added to the respective soil samples in an irrigation-like event (the 'irrigation event'). The soil samples were then drained and incubated in the dark, at 24 °C for one month. Subsamples from the incubated soil samples were taken at day 3, day 7, day 14 and day 31 after the 'irrigation event'. Soil samples were sequentially extracted; each extracting solution was analyzed for concentration of Pi and total P (Pt) and prepared and analyzed for $\delta^{18}O_p$ of Pi.

2.2. Soil samples used in the experiment

Soil (a calcareous alluvial clay soil, Acre, Israel) was obtained in 2006 from two maize growing experimental plots that were fertilized and irrigated differently. In 2002, P was applied to both plots as a basal dressing at a rate of 80 kg Pi ha^{-1} ("Super Phosphate", 21% Pi, FERTILIZERS & CHEMICALS LTD.) along with potassium amendment. Between 2002 and 2006 one plot was irrigated with low grade, secondary RWW, containing high P concentration (Pi = 3–8 mg L^{-1} and total P, Pt = 13–17 mg L^{-1}), resulting in application of ~40 kg P ha^{-1} each season and the second plot was irrigated with FWF. The chemical fertilizer used consisted mostly of N and K with very little P (a common practice in Israel). In 2006, P was applied to the FWF soil via irrigation at a rate of 10 kg Pi ha^{-1} ("Idit series", 35% Pi; FERTILIZERS & CHEMICALS LTD.). The plots were drip irrigated seasonally from May to September at a rate ranging between 400 to 450 mm.

Top soil (0–5 cm) from approximately 50 locations at each of the plots was collected in August 2006, a few days after the last irrigation, and approximately 3 months after the irrigation season begun. Soil samples from each plot were combined, air dried, crashed and sieved using a 2 mm sieve to get a representative homogenized sample. The combined samples representing the soil from the RWW or the FWF treated plots, prior to our experiment are referred to as the "original RWW soil" and the "original FWF soil", respectively.

2.3. The 'irrigation event'

Reclaimed wastewater and freshwater used for irrigating the respective field experimental plots were collected and kept frozen until use in the 'irrigation event'. Filtered reclaimed wastewater (0.45 μm) was analyzed for $\delta^{18}O_p$ (as described below for water extract of soils) and yielded a value of 18.5‰. This value is identical to the $\delta^{18}O_p$ of the water extraction solution of the RWW soil (Zohar et al., submitted). Thus, in order to allow tracking of P applied in the 'irrigation event', the RWW was spiked with $K_2HPO_4*3H_2O$ (SIGMA p-5504 Lot 119H018) such that the resulting $\delta^{18}O_p$ value of the RWW used in the 'irrigation event' was 14.3‰. Freshwater (not containing fertilizer) from Acre, Israel (2.5 L) were mixed with tap water from Santa Cruz, CA (1.5 L), and with 70 mg of NaCl in order to increase volume and achieve a final electric conductivity (597 μS/cm) similar to freshwater from Israel (620 μS/cm); the ratio of Na to Ca and Mg in the water used in the experiment was similar to that applied in the field. The combined 4 L of freshwater was amended with the chemical fertilizer used in the experimental plot in Acre, to achieve a similar concentration of P as that in the RWW used for the 'irrigation event' (see Table 1). The $\delta^{18}O_p$ of FWF used for the 'irrigation event' was

Table 1
The 'irrigation event' — characteristics of applied water, drain water, and P soil retention for each soil column.

	FWF^{+} soil columns				RWW^{++} soil columns	
Column number	1	2	3	4	5	6
Applied water						
Pi (mg L^{-1})	12.8	12.8	12.8	12.8	11.4	11.4
Po[a] (mg L^{-1})	0	0	0	0	5	5
$\delta^{18}O_p$ [b] (‰)	28	28	28	28	14.3	14.3
Drain water						
Pi, (mg L^{-1})	4.3	5.3	4.2	4.2	3	2.9
Average Pi		4.5 (0.5)			2.9 (0.1)	
Po[a], (mg L^{-1})	1.5	2.1	1.5	2.9	1.2	1
Average Po		2.0 (0.7)			1.1 (0.1)	
$\delta^{18}O_p$ [b] (‰)	24.4	25.2	24.4	25.7	14.7	15
Average $\delta^{18}O_p$ (‰)		24.9 (0.6)			14.8 (0.2)	
P retained by the soil[c]						
Pi (mg L^{-1})	8.5	7.5	8.6	8.6	8.4	8.6
Pi (%)	66	59	67	67	74	75

Numbers in parentheses are the standard deviations.
$^{+}$FWF — fresh water with fertilizer; $^{++}$RWW — reclaimed wastewater.
[a] Determined as the difference between total P and SRP for each sample.
[b] Indicates $\delta^{18}O_p$ of Pi.
[c] Determined as the difference between Pi in added irrigation water and Pi in the drain water.

analyzed according to McLaughlin et al. (2004) and had an isotope value of 28‰.

Soil samples (60 g each) were packed in Buchner funnels — 4 funnels (of either 7 cm or 7.5 cm diameter) for the FWF soil and 2 funnels (of 7 cm diameter) for the RWW treatment. A glass wool plug was placed below the soil, and a filter paper (glass fiber) was placed on top of each soil. A vessel below the funnel was positioned to collect the discharged drain water. Since the original FWF soil contains less Pi than the RWW soil, more soil (i.e., 4 funnels) was used for the FWF treatment such that sufficient sample is available for isotopic analysis (Zohar et al., submitted), while still maintaining constant incubation conditions (e.g. water to soil ratio, etc.).

Prior to the 'irrigation event', soil samples were preconditioned in order to enhance microbial activity. Each soil funnel was amended with 25 mL of 40 mg N L^{-1} solution (NH_4NO_3), such that the soil reached water content close to field capacity and soils were left to stand at room temperature for three days.

For the 'irrigation event' one liter of RWW or FWF was added to each funnel using a burette, at a drip rate of about 1.4 cm^3 min^{-1} (i.e. the 'irrigation event' lasted 11.5–12 h). The average water application was 240 mm, which is equivalent to about half a season of field irrigation. The soils were allowed to drain for a day, after which their water content was close to field capacity of 50% (water weight/soil weight). The drain water of each column was collected and Pi and Pt concentrations were determined (see Table 1). The drain solutions were prepared for $\delta^{18}O_p$ analysis according to the protocol of the soil's water extract (see below).

2.4. Soil incubation and sampling

The soil from each funnel was transferred to a glass beaker a day after the 'irrigation event' and incubated for a month in the dark. The ambient temperature of the first 3 days after the 'irrigation event' was ~21 °C and then the temperature was adjusted to 24 °C, similar to soil temperature in the experimental plots, during the summer months, when the soils were sampled. The soil moisture was maintained by partial cover of the beaker with Parafilm, addition of water (milli-Q) every few days and by keeping water vessels in close proximity to the soil beakers in the incubator to maintain high air humidity. Soil moisture was tracked routinely by weighing each soil sample and the data was later used to reconstruct the changing $\delta^{18}O$ of the water molecules through time.

Sampling was done 3, 7, 14 and 31 days after the 'irrigation event'. Before sampling, each soil was mixed. The weight of the subsample collected from each beaker (12–14 g) was determined based on the amount of soil needed to reach the minimum required amount of Pi for preparation of samples for isotopic analysis of the water extract fraction. The obtained soil samples were dried at 24 °C, crashed and each sub sample homogenized in preparation for soil sequential extraction (see below).

2.5. Soil sequential extraction and preparation of samples for isotopic analysis

The combined method of soil sequential extraction and silver phosphate precipitation as reported by Zohar et al. (submitted), was employed. In brief, soil was sequentially extracted by a modified Hedley et al. (1982) procedure (see Fig. 1): 3.3 g of soil was sequentially extracted using 200 mL of each of the following solutions (in order of extraction): DDI water, 0.5 M $NaHCO_3$ pH = 8.5, 0.1 M NaOH, 1 M HCl. The soil was shaken for 16 h with each of the extracting solutions. Soil solution was separated from the solids by centrifugation at 9000 rpm (9056×*g*) for 20 min, followed by vacuum filtration (0.45 μm). Each extraction solution was treated by a modified McLaughlin et al. (2004) procedure to concentrate, purify and precipitate silver phosphate for $\delta^{18}O_p$ analysis as described by Zohar et al. (submitted).

Fig. 1 is a schematic diagram of the above protocol and provides a general description of the relevant chemical characteristics of each extract (adapted from Zohar et al., submitted).

An additional extraction step using hot (80 °C) concentrated HCl to extract occluded P (according to Tiessen and Moir, 1993) was applied to the original soils only (RWW soil and FWF soil, before the experiment). This extract, however, was not analyzed for $\delta^{18}O_p$ since this step involves heating at 80 °C, which may result in isotopic alterations. However, we don't expect this non reactive P pool to be involved in any major P transformation over the time scale of our experiment.

2.6. P concentration determination

Determination of Pi concentration of the water used for the 'irrigation event', the drain water of the soil funnels and the DDI extract of the soils was done according to the Molybdenum blue method, at 880 nm. Pi of the other soil extract solutions was determined by the same method, after pH neutralization.

Pt concentration in the RWW, in the drain water and in the extracted solutions was determined after hydrolization (Tiessen and Moir, 1993): 5 mL of sample solution were digested using 10 mL of 0.9 M sulfuric acid and K-persulfate, in an autoclave for 1 h. K-persulfate was used as follows to account for differences in expected Po in each solution (see Tiessen and Moir, 1993): 0.7 g for the RWW solution; 0.45 g for the drain water and for the water extracted solutions; 0.6 g for the bicarbonate extract and 0.85 g for the NaOH extract solutions. Po was determined by subtracting concentrations of Pi from Pt.

2.7. Mass-spectroscopy analysis

Samples of silver phosphate were weighed (0.35 to 0.45 mg) into silver capsules along with approximately 1 mg of 50% nickelized carbon (Elemental Microanalysis LTD, part nr B1182) and were analyzed by continuous-flow isotope ratio mass spectrometry (CF-IRMS) as described in McLaughlin et al. (2004). The $\delta^{18}O_w$ was analyzed by laser spectroscopy on a Los GatosResearchDLT-100 Liquid-Water Isotope analyzer, using a modification of the method described in Lis et al. (2008). All mass spectrometry analyses were done at the Menlo Park U.S. Geological Survey Stable Isotope Laboratory.

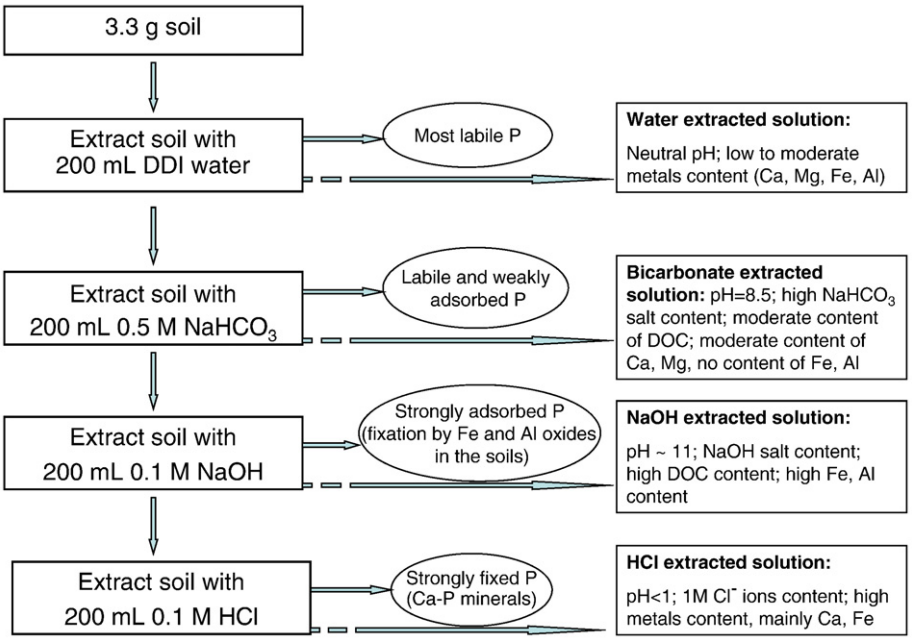

Fig. 1. Soil sequential extraction (modified Hedley et al., 1982), soil P fractions and typical resulting solutions characteristics. It can be assumed that the extracting solutions posses similar characteristics even when employed to different types of soils. It is, however, recommended to know ahead the characteristics of the soil of interest, specifically, Pi concentration, DOC and the metals content for each extracting solution. Knowing these should allow making adjustments to the procedure as needed when preparing samples for isotopic analysis.

Ag_3PO_4 precipitated from each sample was homogenized and analyzed at least 3 times. Standard deviation values presented are for the same Ag_3PO_4 and hence represent mostly the instrument's reproducibility and the degree of sample homogenization. Full duplication of the whole procedure for each subsample was practically not possible, because we did not want to deplete the incubated soil samples. However consistent trends in the isotopic changes with time were observed for each of the soil fractions, thus indicating that the data represent natural trends and not random values.

2.8. Calculations of isotopic mass balance of soil P fractions

Expected $\delta^{18}O_p$ of each soil fraction was calculated by isotopic mass balances, in order to test how the measured $\delta^{18}O_p$ values differ from simple mixing in a case where no biogeochemical isotopic alterations take place. These mass balance calculations use the isotopic composition and concentration of two contributing Pi pools ("pool A" and "pool B") that together make up a new "mixed" pool. Two sets of calculations were done, in both, Pi in the original soil (prior to the irrigation event) constituted pool A ("OS" in Eqs. (2a) and (2b)) and the measured Pi of the incubated soil was regarded as the final new pool ("IS" in Eqs. (2a) and (2b)), thus, the result of each of the calculations is the expected $\delta^{18}O_p$ of the final new pool ("calc-IS" in Eqs. (2a) and (2b)). The first set of calculations used the applied P in the 'irrigation event' as pool B (IW in Eq. (2a)) and the second set of calculations used the water extracted Pi (DDI-Pi) concentration and isotope value of the same sampling time, which represents the soil solution, as pool B (DDI in Eq. (2b)). Eq. (2b) was applied only for soil fractions bicarbonate, NaOH and HCl; calculations were not done for the DDI extract itself as that data cannot be used as both a mixing end member and a final mixture.

$$\frac{[Pi]_{os}}{[Pi]_{IS}} \times (\delta^{18}O_p)_{OS} + \frac{[Pi]_{IS}-[Pi]_{OS}}{[Pi]_{IS}} \times (\delta^{18}O_p)_{IW} = (\delta^{18}O_p)_{calc-IS} \quad (2a)$$

$$\frac{[Pi]_{OS}}{[Pi]_{IS}} \times (\delta^{18}O_p)_{OS} + \frac{[Pi]_{IS}-[Pi]_{OS}}{[Pi]_{IS}} \times (\delta^{18}O_p)_{DDI} = (\delta^{18}O_p)_{cala-IS} \quad (2b)$$

3. Results

3.1. Concentration and isotopic composition of soil P prior to the experiment and following the 'irrigation event'

The original RWW soil shows higher concentration of P than the original FWF soil for the first four steps of soil extraction (Table 2). The fifth pool, extracted by hot and concentrated HCl, is identical in both soil samples (Table 2), suggesting that this soil fraction likely retains P from early stages of the soil genesis, or at least from time before the field experiment had begun (i.e. in 2002, before the plots were treated differently) and does not respond considerably to the different irrigation treatment of the plots. Higher concentrations of P in all other soil fractions of the original RWW soil are consistent with higher applications of P via RWW irrigation since 2002. The difference in Pi concentration for each of the different soil fractions of RWW soil relative to FWF soil (i.e. RWWs/FWFs difference factor) decreased along the extraction sequence with greater difference for the more labile fractions and smaller differences in the least labile fractions.

Table 2
Pi, Po and Pt concentrations and difference factor for the different extractions of the original soils.

Extracting solution	Original RWW^{+} soil			Original FWF^{++} soil			Pi RWWs/FWFs
	Pi	Po	Pt	Pi	Po	Pt	
	mg P Kg^{-1} soil						
DDI	43.1 (2.5)	1.1	44.2 (4.5)	10.5 (0.8)	4.3	14.9 (4.2)	4
0.5M $NaHCO_3$	68.8 (4.8)	1.1	69.9 (3.9)	33.8 (2)	1.3	35.1 (3)	2
0.1M NaOH	54.2 (1.8)	11.8	66 (5.6)	29.8 (3.4)	8.6	38.4 (4.9)	1.8
1M HCl	215.8 (27)	0	215.8 (27)	205.4 (19.4)	0	205.4 (19.4)	1.05
conc. Hot HCl	124.6 (13.1)	19.7	144 (4.3)	126.8 (10.6)	19.4	146 (7.2)	0.98

Numbers in parentheses are the ±standard deviations (n = 7 to 10). Po = Pt–Pi.
$^{+}$RWW — reclaimed wastewater; $^{++}$FWF — fresh water with fertilizer.

Table 3
Pi concentrations of soil extracts during the incubation experiment for RWW and FWF treated soils.

Extracting solution	RWW soil				FWF soil			
	Time, day							
	3	7	14	31	3	7	14	31
DDI (mg Pi kg^{-1} soil)	89	103	105	95	68	60	70	69
	(8.9)	(0.3)	(3.1)	(1)	(4.7)	(5.4)	(5.6)	(4.1)
Increase[a]	106%	140%	144%	120%	547%	471%	566%	557%
0.5M $NaHCO_3$ (mg Pi kg^{-1} soil)	93	90	117	122	76	70	85	83
	(1.9)	(8.1)	(7)	(13.4)	(1.5)	(11.9)	(5.1)	(3.3)
Increase[a]	35%	31%	70.5%	78%	125%	107%	151%	145%
0.1M NaOH (mg Pi kg^{-1} soil)	70	76	70	81	58	63	57	55
	(3.5)	(6.1)	(2.8)	(0.8)	(4.6)	(4.4)	(3.4)	(5)
Increase[a]	29%	40%	29%	49%	95%	111%	91%	85%
1 M HCl (mg Pi kg^{-1} soil)	242	241	249	243	240	254	250	239
	(4.8)	(6.3)	(7.8)	(2.6)	(7.2)	(6.3)	(9.3)	(8.8)
Increase[a]	12%	12%	15%	13%	17%	24%	22%	16%

Numbers in parentheses are the ± standard deviations (n = 4 to 3).
Po = Pt–Pi. Day 3, day 7, day 14 and day 31 refer to time after the 'irrigation event'.
[a] Percent increase relative to the original soil.

Following the 'irrigation event' at the beginning of the experiment, P concentrations in all soil fractions for both soil samples increased compared to the original soil concentrations. These concentrations change with time (during the incubation) but remain higher than initial (pre irrigation) values for the DDI, $NaHCO_3$ and NaOH extracts of both soil samples throughout the incubation month. The Pi concentrations of these fractions in the RWW soil are also maintained higher compared to the FWF soil throughout the incubation month (Table 3). Po concentrations are presented graphically in Fig. 2. $\delta^{18}O_p$ of all soil extracts we analyzed also changed along the incubation following the irrigation with RWW or FWF (Table 4).

3.2. Isotopic mass balance calculations

Table 5, a and b (for RWW soil and FWF soil, respectively), present the results of the first set of mass balance calculations using Eq. (2a). This set of calculations assumes mixing of the initial P in the original soil (pool A) and the applied P in the 'irrigation event' (contributing pool B). This calculation predicts the expected impact of the applied Pi on the various Pi pools in each fraction of the two soil samples, assuming simple mixing and no biological activity which changes the isotope ratios. Table 5, c and d (RWW soil and FWF soil, respectively), present a second set of mass balance calculations using Eq. (2b). Here we assume

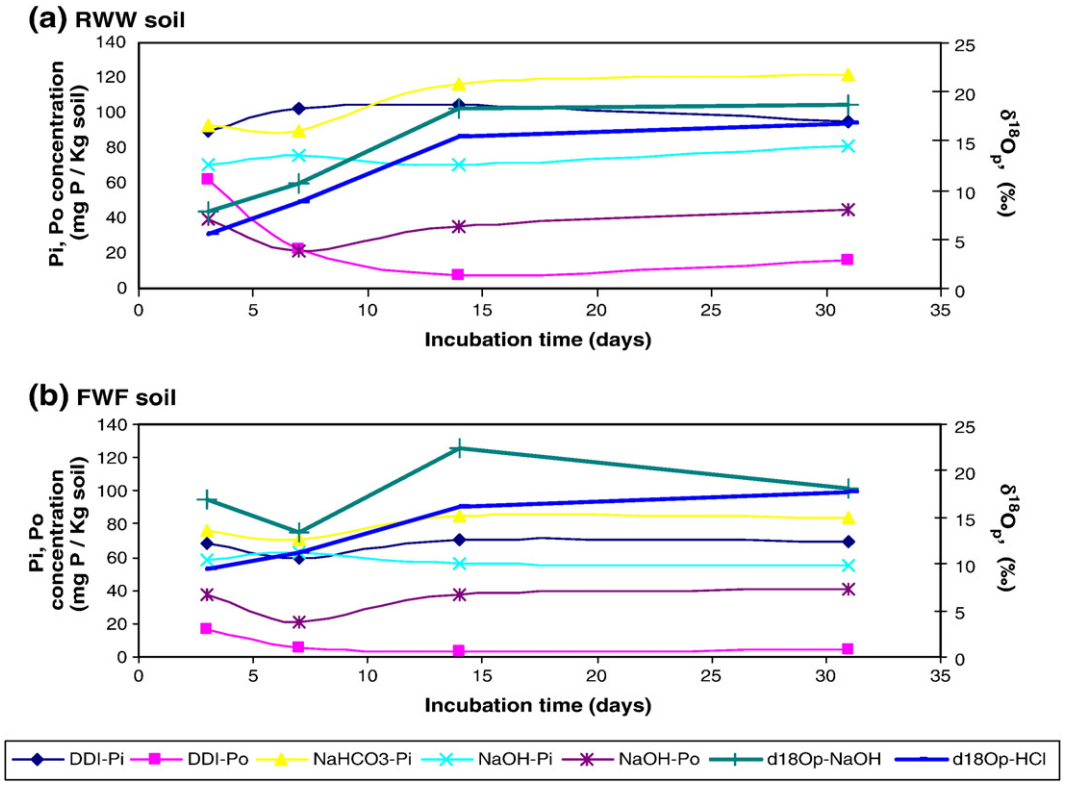

Fig. 2. (a) RWW soil, (b) FWF soil: Pi and Po concentrations of the various soil fractions together with NaOH–$\delta^{18}O_p$ and HCl–$\delta^{18}O_p$, as were measured along the month of incubation; HCl–Pi and bicarbonate–Po were excluded to focus on the more reactive soil fractions.

Table 4
Oxygen isotopic composition of Pi of the original soils extracts, of the RWW and FWF solutions applied in the 'irrigation event' and of the soil extracts during the incubation experiment.

		$\delta^{18}O_p$ of soil extraction solutions and of the applied P (‰)							
		RWW soil				FWF soil			
		DDI	$NaHCO_3$	NaOH	HCl	DDI	$NaHCO_3$	NaOH	HCl
Time before or after the 'irrigation event'	Original soil	18.5 (0.6)	20.3 (0.2)	9.1 (0.2)	6.3 (0.1)	10.7 (1)	19 (0.9)	7.8 (0.5)	7.7 (0.7)
	Applied P		14.3 (0.1)				28 (0.5)		
	3 days	17.6 (0.5)	20 (1)	7.8 (0.9)	5.6 (0.9)	24.5 (0.2)	22.2 (0.2)	17 (0.8)	9.4 (1.6)
	7 days	18.4 (0.3)	19.9 (0.4)	10.7 (1)	8.7 (0.1)	21.7 (0.9)	22.6 (0.6)	13.3 (0.9)	11.2 (0.8)
	14 days	19.4 (0.5)	19.5 (0.3)	18.3 (1)	15.5 (0.5)	21.9 (0.1)	23.7 (0.5)	22.5 (0.2)	16.1 (0.6)
	31 days	24.1 (0.1)	21 (0.1)	18.7 (0.4)	16.8 (0.5)	20.3 (0.2)	22.9 −0.3	18.1 (0.4)	17.8 (0.7)

Numbers in parentheses are the ± standard deviations (‰) for n ≥ 3.

P in the various soil fractions is controlled by mixing of the P in the original soil fractions (pool A) and Pi from the soil solution which is assumed to be represented by the DDI extracted fraction (i.e., measured DDI–$\delta^{18}O_p$ of the same sampling occasion as contributing pool B). This calculation evaluates the impact of the Pi dissolved in soil water on the other soil P fractions, assuming that this most labile fraction is the pool most available for interacting with other less labile pools. This set of calculations cannot be done for the DDI fraction itself. Deviation of the measured $\delta^{18}O_p$ from the expected $\delta^{18}O_p$ (calculated using either set of equations), for each soil fraction, indicates the involvement of biogeochemical processes that include isotopic alterations.

The extent of the biogeochemical transformations is proportional to the degree of deviation from expected results of the mixing scenarios in Table 5 a, b, c, d.

4. Discussion

Changes observed in soil P concentrations and isotope values during the month of incubation may be explained by one or more of the following processes: 1. Addition and mixing with the applied P of the 'irrigation event' (as predicted by the calculated values in Table 5 a, b); 2. Exchange or addition of P dissolved in soil solution with other soil P fractions (as predicted by the calculated values in Table 5 c, d); 3. Isotope equilibrium effects as expected from PPase enzymatic activity; 4. Isotopic non equilibrium effects (e.g. kinetic effects) induced by extracellular enzymatic activity or P uptake. Processes of Pi adsorption and/or precipitation may involve isotopic alterations as well; however, isotopic imprint associated with these processes is relatively small and may not be easily identified. The involvement of the above processes in determining the $\delta^{18}O_p$ of each of the analyzed soil P fractions is discussed below.

Table 5
Isotopic mass balance calculations for the soil fractions, along the incubation month.

	Day 3		Day 7		Day 14		Day 31	
	Calc	Meas	Calc	Meas	Calc	Meas	Calc	Meas
a. $\delta^{18}O_p$ (‰)–RWW soil								
DDI	16.3	17.6	16.1	18.4	16	19.4	16.2	24.1
0.5M $NaHCO_3$	18.8	20	18.9	19.9	17.8	19.5	17.7	21
0.1M NaOH	10.3	7.8	10.6	10.7	10.3	18.3	10.8	18.7
1 M HCl	7.2	5.6	7.1	8.7	7.4	15.5	7.2	16.8
b. $\delta^{18}O_p$ (‰)–FWF soil								
DDI	25.3	24.5	24.9	21.7	25.4	21.9	25.3	20.3
0.5M $NaHCO_3$	24	22.2	23.6	22.6	24.4	23.7	24.3	22.9
0.1M NaOH	17.6	17	18.4	13.3	17.3	22.5	17.1	18.1
1M HCl	10.6	9.4	11.6	11.2	11.3	16.1	10.6	17.8
c. $\delta^{18}O_p$ (‰)–RWW soil								
0.5M $NaHCO_3$	19.6	20	19.8	19.9	19.9	19.5	22	21
0.1M NaOH	11	7.8	11.7	10.7	11.4	18.3	14	18.7
1 M HCl	7.5	5.6	7.6	8.7	8.1	15.5	8.3	16.8
d. $\delta^{18}O_p$ (‰)–FWF soil								
0.5 M $NaHCO_3$	22.1	22.2	20.4	22.6	20.7	23.7	19.7	22.9
0.1 M NaOH	15.9	17	15.1	13.3	14.5	22.5	13.6	18.1
1 M HCl	10.1	9.4	10.4	11.2	10.2	16.1	9.5	17.8

Calc = calculated; meas = measured.

4.1. Water extractable and most labile Pi–DDI extraction

Pi concentration in the most labile fraction of the soil, the water extractable Pi (DDI-Pi), is four times higher in the original RWW soil than in the original FWF soil (Table 2); the concentration in the FWF soil's water extract (10.5 mg Pi kg^{-1} soil) is typical to low P soils (Sibbesen and Sharpley, 1997).

Pi and Po concentrations increased dramatically in both soils following the 'irrigation event' (Table 3, Fig. 2). Although Pi concentrations in the applied solutions were very similar (RWW 11.4 mg Pi L^{-1} and FWF 12.8 mg Pi L^{-1}) and Pi which was retained by the soils was the same (~8.5 mg Pi L^{-1} corresponds to ~142 mg Pi kg^{-1} soil, Table 1), throughout the incubation month, the size of the DDI-Pi pool in the RWW soil was larger than that in the FWF soil (89–105 and 60–70 mg Pi kg^{-1}, respectively).. This indicates that applying excess P is likely to result in increase in labile P in both soil samples and loss and transport to waterways. Measured Po concentration in the DDI extract was also higher in the RWW soil (62 mg Po kg^{-1} soil) compared to the FWF soil (17 mg Po kg^{-1} soil), however both soils exhibited a significant decrease in Po concentration in the DDI extractable soil pool with time (Fig. 2). This change suggests that Po in the soil solution was hydrolyzed to Pi or that Po from the soil solution was lost by adsorption or transformation to other P pools in the soil.

After applying RWW with $\delta^{18}O_p$ of 14.3‰ to the RWW soil in the 'irrigation event', the $\delta^{18}O_p$ of the DDI–Pi pool exhibited a slight decrease from 18.5‰ in the original RWW soil to 17.6‰; this decrease was followed by a continuous increasing trend throughout the incubation period (from +17.6‰, to +24.1‰, Table 4). The measured value at day 3 (+17.6‰) was slightly more positive than the calculated one (+16.3‰, Table 5a) but depleted compared to the original value, possibly reflecting a small impact of the depleted Pi in the RWW used in the "irrigating event". However, for the rest of the sampling intervals, $\delta^{18}O_p$ values were different from those expected from simple mixing of the original soil solution Pi (as indicated by the P pool extracted by DDI) with the applied RWW (see comparison in Table 5a), hence, other processes involving biogeochemical isotope effects must have taken place in the soil solution to result in the measured $\delta^{18}O_p$ values.

Pi in the soil solution may originate from release of intracellular Pi into the environment during active growth of soil microorganisms (Blake et al., 2005). Intracellular enzyme activity will result in Pi with $\delta^{18}O_p$ that is in isotopic equilibrium with soil water at the soil temperature. If this process is prevalent and impacts the DDI–Pi then measured $\delta^{18}O_p$ is expected to be close to that predicted from isotopic equilibrium and changes over time will reflect predominantly the changes of $\delta^{18}O_w$ of the soil solution, during the incubation month (temperature was held constant). The $\delta^{18}O_w$ of the initially applied RWW (−2.7‰) would change over time due to dilution by Milli-Q water additions (−5.4‰, depletion effect) and due to water loss (enrichment during evaporation). Evaporative enrichment can be calculated using water-vapor fractionation in a closed system (Criss, 1999):

$$\delta = \delta_0 + \varepsilon^*(1-f) \quad (3)$$

where δ is $\delta^{18}O_w$ and δ_0 is the initial water composition, ε is the fractionation factor (water-vapor) and f is the fraction of the residual water pool. Calculating $\delta^{18}O_w$, taking evaporation and dilution effects into account, yields an enrichment trend with time (indicating that evaporation was the dominant effect) and a corresponding enrichment trend of the calculated equilibrium $\delta^{18}O_p$. The calculated equilibrium $\delta^{18}O_p$ values (until day 28 of incubation) along with the measured values of the DDI–Pi of the RWW soil are shown in Fig. 3a. The measured DDI–$\delta^{18}O_p$ and the calculated equilibrium $\delta^{18}O_p$ both exhibit a linear increase trend ($r^2 = 0.98$ and $r^2 = 0.99$, respectively) with incubation time and agree well with each other. For the first 3 sampling times, the measured values are slightly lower than the equilibrium values, while at day 31 they are slightly higher than expected extrapolated equilibrium, even when considering analytical errors. The lower than equilibrium $\delta^{18}O_p$ could be explained by some contribution of depleted Pi produced by extracellular mineralization of Po compounds, as expected from the decrease in Po over time (Liang and Blake, 2006, see also discussion of NaOH and HCl soil fractions below). A small residual contribution from the applied Pi of +14.3‰ at the 'irrigation event' may also explain this offset and the decrease in difference over time, as the effect of this residual signature is gradually erased as a result of increasing biological turnover. Alternatively, the calculated equilibrium $\delta^{18}O_p$ which used the evaporation corrected $\delta^{18}O_w$ values could be higher than the measured values due to overestimation of the evaporation effect. Eq. (3) applies to a well mixed system, where a complete water reservoir participates in the evaporation, yielding a water vapor with a specific $\delta^{18}O_w$ enrichment. This enrichment could be higher than one achieved by evaporation of water from the outer water films of soil aggregates, during their incubation. In the latter case, the outer part may dry faster than the inner part and the overall evaporation is actually less than that expected from the average water content of the aggregates (i.e., non-mixed system). Accordingly, the actual $\delta^{18}O_w$ enrichment may be lower than expected from Eq. (3) and account for the difference between the actual $\delta^{18}O_p$ and calculated equilibrium values. Direct analysis of soil water $\delta^{18}O_w$ and measurement of enzyme activities in soil solution could be used to confirm or refute these interpretations.

The measured DDI–$\delta^{18}O_p$ at day 31 is +24.1‰ while extrapolation of the first three values yields an expected value of +22.1‰ (not shown). Interestingly, extrapolation of the calculated equilibrium $\delta^{18}O_p$ trend for the first 28 days to day 31 predicts a similar value (22.6‰). The 2‰ positive shift of the measured value on day 31, when compared to values extrapolated from both measured and calculated equilibrium trends, may be attributed to slower P recycling rates a month after RWW application. Lower rates of P cycling and turnover will result in more pronounced impact of processes that deviate from equilibrium. Specifically, preferential uptake of depleted Pi by the biota could result in enriching the residual Pi pool, the soil solution, in this case (Blake et al., 2005).

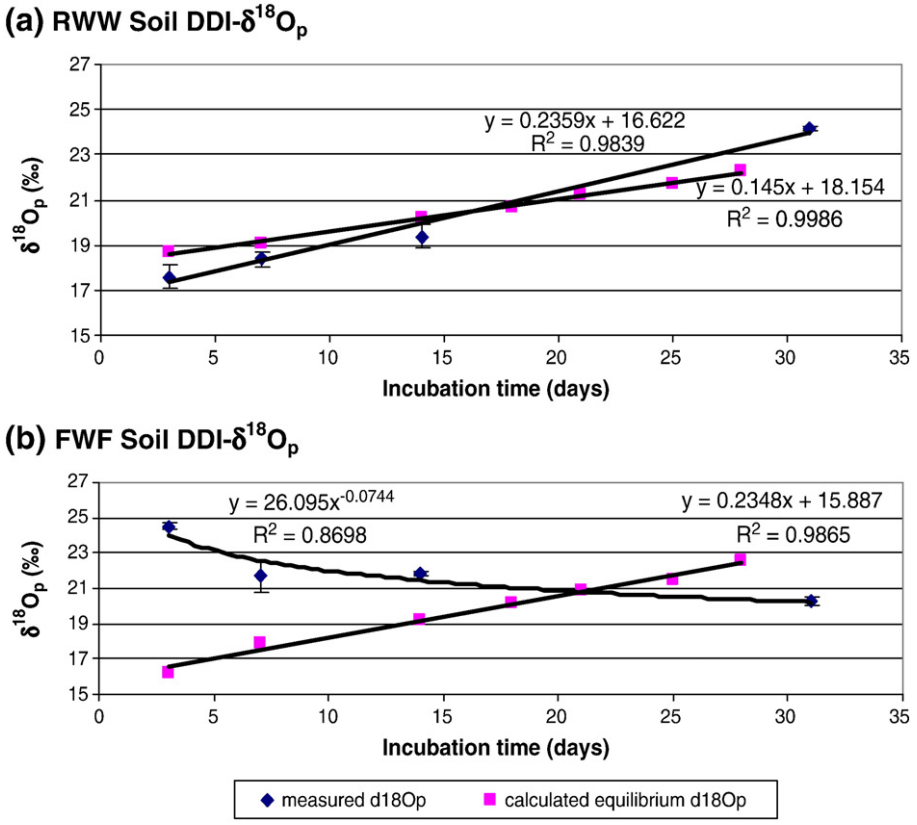

Fig. 3. (a) RWW soil, (b) FWF soil: measured DDI–$\delta^{18}O_p$ (diamonds) and calculated $\delta^{18}O_p$ according to equilibrium value (squares). Error bars of the measured $\delta^{18}O_p$ represent standard deviation for $n \geq 3$. The equilibrium $\delta^{18}O_p$ was calculated according to Longinelli and Nuti (1973), using calculated $\delta^{18}O_w$ of soil. Note — the best fit trend line for measured $\delta^{18}O_p$ is linear in (a) and logarithmic in (b).

Based on the above discussion we conclude that the DDI–$\delta^{18}O_p$ of the RWW soil was controlled primarily by equilibrium enzymatic effects with other processes exerting secondary effects. Assuming similar processes occur in the field, this implies that the DDI–$\delta^{18}O_p$ of the original RWW soil as was sampled in the field (18.5‰), also primarily reflects a state of enzymatic equilibrium and not the signature of the irrigation water Pi (see discussion in Zohar et al., submitted).

The original FWF soil DDI–$\delta^{18}O_p$ (10.7‰, Table 4), does not reflect the $\delta^{18}O_p$ of the fertilizer used in the plot during the sampling season (28‰). This could be due to the small amount of applied P which is taken up rapidly by the crops without leaving its signature in the soil. The expected $\delta^{18}O_p$ at equilibrium (15.4‰, 24 °C, $\delta^{18}O_w = -4.9$‰) is also different from the measured value. Based on the relative similarity of the $\delta^{18}O_p$ of the DDI extract and the HCl extract (7.7‰) of the original FWF soil, one explanation could be that the solubility of P mineral phases (extracted with HCl) exerts the primary control on the $\delta^{18}O_p$ of the soil solution in the field setting. The low Pi concentration in the DDI extract solution (0.16 mg L^{-1}, i.e., 10 mg Pi kg^{-1} soil, Table 2) further supports this, as it agrees with data presented by Lindsay (1979) for solubility of stable forms of calcium phosphate in neutral pH soils when in geochemical equilibrium. Stable Ca-phosphate minerals are expected to dominate the P mineral phase in the FWF soil (Morgan, 1997). Physical separation and direct analysis of the isotopic composition of mineral phases in the soil will enable further confirmation of this interpretation. Alternatively, it is possible that the soil P fractions with low isotope values (DDI–Pi, HCl–Pi and NaOH–Pi) are all controlled by the same biogeochemical processes (see below), leading to the measured low values.

After the 'irrigation event' the impact of the irrigation FWF water Pi (28‰) is clearly seen in the FWF soil's DDI–$\delta^{18}O_p$, (i.e., the values increases dramatically and are closer to the applied values than to the original ones). The initial impact of the added fertilizer, however, decreased considerably with time as seen by the comparison between the mixing mass balance calculated values and measured values (Table 5b). Three days after application, DDI–$\delta^{18}O_p$ is +24.5‰, and continued to decrease, while the expected mass balance using the added fertilizer as a mixing end member remains ~25‰. The depletion trend of the measured values may be explained by partial biological cycling of P, which shifts the soil solution (mixed original and applied Pi) $\delta^{18}O_p$ toward isotopic equilibrium. The expected equilibrium values were lower at the beginning of the incubation month, but increased with time (Fig. 3b) like in the RWW soil sample. At the end of the incubation month, the equilibrium values were closer to the measured values than at the start of the incubation. At day 31 the measured $\delta^{18}O_p$ value is 20.3‰, while the equilibrium $\delta^{18}O_p$ (based on trend from previous days data, $r^2 = 0.98$) is 23.2‰.

Our results for the P pool extracted by DDI indicates that throughout the incubation month, the dissolved Pi of the FWF soil solution is not in isotopic equilibrium while that of the RWW is closer to equilibrium; this suggests that biological activity in the FWF soil sample is slower than in the RWW soil sample.

4.2. Loosely bound Pi–Bicarbonate extraction

P extracted by bicarbonate is considered relatively labile and consists of P loosely bound to clay minerals and to OM by cation bridges (e.g., Ca^{2+}, Mg^{2+}), (Tiessen and Moir, 1993). Thus, P concentration associated with this pool is likely determined by availability of adsorption sites and reflect their rate of saturation. The original RWW soil and FWF soil bicarbonate extract contain ~69 mg Pi kg^{-1} soil and ~34 mg Pi kg^{-1}soil, respectively (Table 2). After application of P in the 'irrigation event', the bicarbonate Pi concentration of both soils increased. This could be attributed to occupation of existing free adsorption sites, as well as to new adsorption sites created when cations (e.g., Ca), introduced to the soils by the freshly applied solutions, serve as bridging cations on soil surfaces (e.g., oxides, Barrow, 1972). Three days after irrigation, Pi concentrations increased but did not change in the following sampling time at day 7 (~90 mg Pi kg^{-1} soil and ~75 mg Pi kg^{-1} soil, respectively). In both soil samples, a further increase in the bicarbonate–Pi concentration was detected at day 14 and remained relatively the same at day 31 (the average concentrations in days 14 and 31 are ~120 mg Pi kg^{-1} soil and ~84 mg Pi kg^{-1} soil, respectively, Table 3). The increase in concentration in the first two weeks of the incubation period coincides with changes in the Po pool. Soil Po went through hydrolization processes during the first two weeks, as shown by a decrease in Po concentrations (Fig. 2). Soil Po is part of the organic entity of the soil; therefore, we assume the majority of the OM hydrolization in the soil has occurred during that period, as well. Since soil micro-aggregates include clay-OM complexes (Carter, 1996), degradation of OM may allow otherwise occluded clay surfaces and other adsorption sites to become available for Pi adsorption and can explain the stepwise increase in Pi concentration of the bicarbonate extractable soil fraction. It should be noted that, although bicarbonate may potentially extract loosely bound Po as well as Pi, the Po concentrations of this extract, in the original soils and in the incubated soils, are negligible; only in day 7 a small increase relative to the original soils (to ~11.5 mg Po kg^{-1} soil) is detected for both soil samples. This small and transient increase in bicarbonate–Po may be attributed to OM hydrolysis, which yielded Po species as intermediate products (e.g. Colman et al., 2005), followed by hydrolysis to orthophosphate. For example, Taranto et al. (2000) found that RNA degradation increases soil bicarbonate extracted Po and this might have happened in our samples as well.

The $\delta^{18}O_p$ values of the bicarbonate extract of the original RWW and FWF soil samples are quite similar, 20.3‰ and 19.0‰, respectively. This appears to imply that when given sufficient time (e.g. at least 3 months in the field), the same biogeochemical processes impact this fraction in both plots and results in $\delta^{18}O_p$ within a narrow range of values. Along the incubation month, $\delta^{18}O_p$ values of bicarbonate extract of the RWW soil remained within 1‰ of the bicarbonate extract of the original soils (~19.5–21‰). A very good agreement is found between the measured RWW soil bicarbonate–$\delta^{18}O_p$ values and those presented in Table 5c, which were calculated using mixing mass balance based on DDI–$\delta^{18}O_p$ of the same sampling occasion. This suggests that the soil solution Pi (represented by the DDI extractable Pi) in the RWW soil is in constant interaction and exchange with the loosely bound Pi (bicarbonate extractable) and that the latter therefore indirectly reflects the soil's biological activity. The bicarbonate–$\delta^{18}O_p$ values of the FWF soil sample were higher than those in the original FWF soil sample: $\delta^{18}O_p$ of day 3 (22.2‰) is in good agreement with the calculated value based on DDI–$\delta^{18}O_p$ (22.1‰, Table 5d), $\delta^{18}O_p$ of the subsequent sampling (day 7) is practically the same (22.6‰), while the calculated value is slightly lower (20.4‰, Table 5d). Enrichment is detected at day 14 (23.7‰) concurrent with an increase in Pi concentration. This enrichment cannot be explained by direct adsorption or exchange with soil dissolved Pi at day 14 since the DDI extracted Pi had a lower value and thus its direct contribution to the loosely bound pool would yield lower $\delta^{18}O_p$ (20.7‰, Table 5d), not the higher value observed. We note, however, that at day 14 significant enrichment is recorded also for the NaOH–$\delta^{18}O_p$ (Table 4, Fig. 2), and it is possible that the same source/process affected both soil fractions (see below).

We conclude that the RWW soil bicarbonate–$\delta^{18}O_p$ in our experiment is primarily controlled by rapid incorporation of Pi from the DDI–Pi pool. However, in the FWF soil the bicarbonate fraction was controlled by interaction with the DDI–Pi pool only at the beginning of the incubation and it appears that later on, Pi exchange with the soil solution was overshadowed by what seems to be non-equilibrium effects as discussed below for the fixed Pi fractions.

4.3. Fixed Pi by oxides and minerals — NaOH and HCl extraction

The soil P fractions extracted by NaOH and HCl are discussed here together since they were found to share similar trends in $\delta^{18}O_p$ for both soils (Table 4, Fig. 2) suggesting that these soil fractions are controlled by similar biogeochemical processes in both soils.

The NaOH–Pi soil fraction increased following P application (i.e., via the 'irrigation event') from 54 mg Pi kg^{-1} soil to 70–80 mg Pi kg^{-1} soil in the RWW soil and from 30 mg Pi kg^{-1} soil to 55–63 mg Pi kg^{-1} soil in the FWF soil. The increase in NaOH–Pi could be related to higher occupation of P adsorption sites (Fe and Al sesquioxides and hydroxides). The HCl–Pi concentrations of the RWW and FWF soils increased to a lesser extent relative to the original soils (from 215 mg Pi kg^{-1} soil to 241–249 mg Pi kg^{-1} soil and from 205 mg Pi kg^{-1} soil to 239–254 mg Pi kg^{-1} soil, respectively). Based on this, one might deduce that the soil fraction extracted by HCl, which represents the most strongly bound P among the four tested soil fractions, is not reactive at all and that other than a small addition of Pi immediately following application, this fraction has not changed. However, the change in the isotopic composition during the incubation month reveals that this soil fraction has gone through considerable dynamics.

As presented in Table 4 and Fig. 2, three days after irrigation, the RWW soil's NaOH–$\delta^{18}O_p$ and HCl–$\delta^{18}O_p$ decreased from 9.1‰ to 7.8‰ and from 6.3‰ to 5.6‰, respectively. These changes cannot be explained by isotope changes expected from simple adsorption or precipitation of the freshly applied P (14.3‰), according to the calculations presented in Table 5. In contrast the impact of irrigation Pi (28‰) is evident in the FWF soil, three days after its application: the $\delta^{18}O_p$ of the NaOH increased from 7.8‰ to 17.6‰, which is practically identical to the mass balance value (Table 5b); the HCl–$\delta^{18}O_p$ increased from 7.7‰ to 9.4‰, which is only slightly lower than the calculated value (10.6‰, Table 5b). From day 3 on, $\delta^{18}O_p$ of the RWW soil NaOH and HCl P fractions and the FWF soil HCl P fraction increased significantly, mainly within the first 14 days. The NaOH–$\delta^{18}O_p$ exhibited the same sequence described above (depletion followed by enrichment) but much larger fluctuations are detected. Finally, at the end of the incubation month the $\delta^{18}O_p$ of both fractions of both soil samples all converged into a narrow range of values (~17‰ to 18‰). Except for the FWF soil at the beginning of the incubation, the trends in $\delta^{18}O_p$ of the NaOH and HCl of both soils along the month cannot be explained by the mixing mass balance values presented in Table 5, indicating that neither the applied P nor the Pi in the soil solution directly impacted these fractions. In addition, the measured values are not consistent with equilibrium values. This suggests that processes that result in non equilibrium effects influence these fractions.

Very similar trends of $\delta^{18}O_p$ (i.e., depletion followed by enrichment) were observed by Blake et al. (2005) when intact *E. coli* cells were grown in pure culture (Fig. 4). Initial depletion in $\delta^{18}O_p$ during the first 80 h of experiment, was associated with increase in the concentration of Pi (orthophosphate was the only P source), and a slight increase in microbial growth (as measured by optical density, Fig. 4 a). During the next 90 to 250 h, the $\delta^{18}O_p$ of Pi in solution increased while its concentrations decreased. The optical density indicated exponential growth has occurred at that time frame. Similar microbial process may take place in soils (lag, exponential and sustainability growth phases of the soil microbial populations; Blagodatsky et al., 2000). While we did not conduct any biological assays, based on the similarity in isotopic trends to those observed by Blake et al. (2005), we suggest that enzymatic processes as those measured in the *E. coli* experiment, took place in our soil incubation. Specifically, the growing microbial community of cells attached to the soil oxide and mineral surfaces could change the phosphate isotopic composition of the NaOH and HCl soil fractions.

Decrease in the dissolved Po concentration (DDI-Po), likely due to microbial degradation of dissolved OM (DOM) in the first two weeks of incubation, correlates with the enrichment of NaOH–$\delta^{18}O_p$ and HCl–$\delta^{18}O_p$ of the RWW soil sample (correlation coefficients of −0.87 and −0.89, respectively) and to the HCl–$\delta^{18}O_p$ of the FWF sample (−0.8), (Fig. 2). Changes in NaOH–Po concentrations in both soils are indicative of microbial activity in those P pools as well, although the trend of change was not as consistent. Degradation of Po, through extracellular phosphomonoesters hydrolization, which involves isotopic fractionation of −10‰ to −30‰ depending on specific enzyme used (Liang and Blake, 2006) can explain the depletion of NaOH–$\delta^{18}O_p$ and HCl–$\delta^{18}O_p$ for the first 3 days (and in the FWW soil's NaOH at day 7). Phosphomonoesters adsorbed by soil oxides were found to be the sole Po species in both original soil samples (^{31}P NMR experiments in our lab, data not shown). Mineralization of adsorbed Po either directly on the soil surfaces followed by immediate uptake or by initial release of Po by-products to the solution followed by mineralization and assimilation at a later stage (Stroud et al., 2007), can explain the data obtained in our experiment. The hydrolysis of freshly added Po by extracellular enzymes will result in production of depleted Pi which will immediately be sequestered by strong adsorption onto unoccupied sites on oxides and as precipitates (e.g., Ca–P minerals), (NaOH and HCl P fractions, respectively) with minimal interaction with the soil's more labile Pi pools. Any depleted Pi formed in this process, which is not immobilized, will get to the solution, be rapidly recycled and impacted by equilibrium effects.

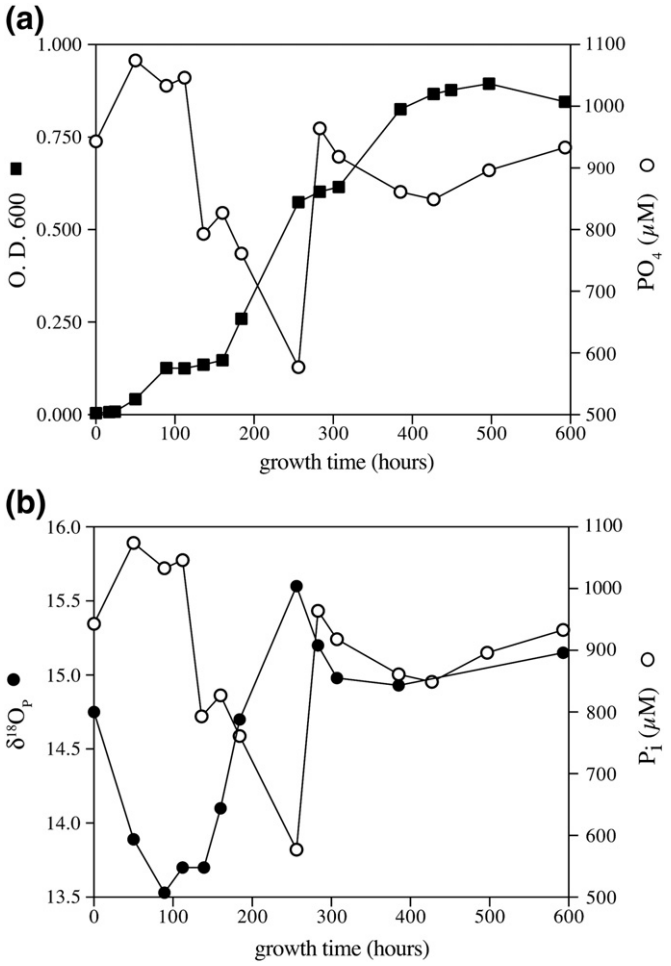

Fig. 4. Results of experiments on Pi uptake by intact E. coli cells grown on inorganic phosphate (Pi) minimal medium at 37 °C (adapted from Blake et al., 2005). O.P. 600 - Optical density at 600nm.

Degradation of Po does not seem to occur in order to fulfill the biomass Pi requirements, since dissolved Pi, which is more bioavailable, was present at high concentrations in both soil samples. Indeed it has been noted that the activity of the bacterial extracellular enzymes, APase and 5′-Nucleotidase, does not always depend on ambient Pi concentration (Chrost, 1991; Ammerman and Azam, 1991). It is likely that the degradation of Po compounds was carried out to provide C metabolites or other limiting constituents (e.g. N), for the growing microbial biomass, as was suggested by others (Tiessen and Moir, 1993; Colman et al., 2005; Liang and Blake, 2006). In soils, synthesis activity is associated with preliminary respiration, before actual biomass increase (Blagodatsky et al., 2000) and thus, OM degradation occurrence in the first 3 days of incubation, likely corresponds to the initial lag phase of biomass growth.

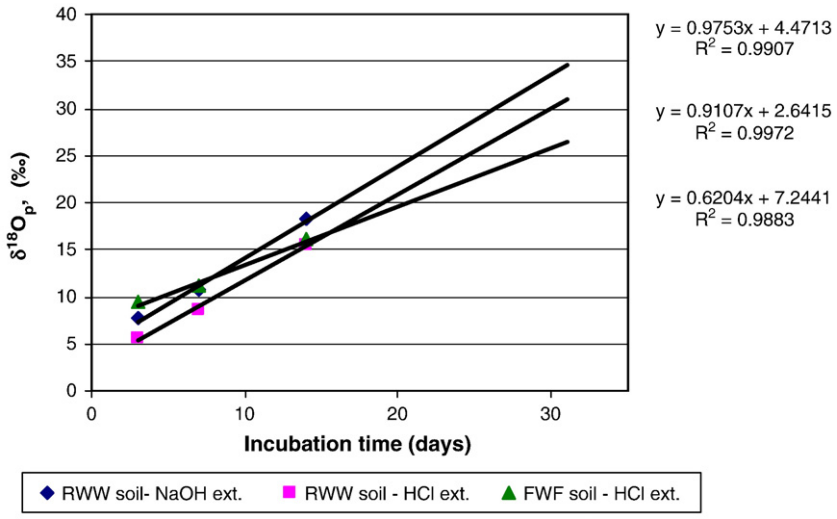

Fig. 5. Enrichment of the RWW soil's NaOH–$\delta^{18}O_p$ (diamonds) and HCl–$\delta^{18}O_p$ (squares) and of the FWF soil's HCl–$\delta^{18}O_p$ (triangles), based on the first three sampling periods. Trend line equations are in the order (from top to bottom): RWW soil–NaOH, RWW soil–HCl, FWF soil–HCl. The FWF soil–NaOH–$\delta^{18}O_p$ is not presented here because its isotopic alterations did not follow a consistent trend.

Strong enrichment of NaOH–$\delta^{18}O_p$ and HCl–$\delta^{18}O_p$ followed the initial depletion and was recorded between day 3 and day 14 of incubation, except for the NaOH–$\delta^{18}O_p$ of the FWF soil sample, where enrichment was only recorded at day 14. This isotopic increase is not accompanied by significant concentration changes. Isotopic enrichment of a residual P pool can occur due to preferential assimilation of isotopically depleted Pi

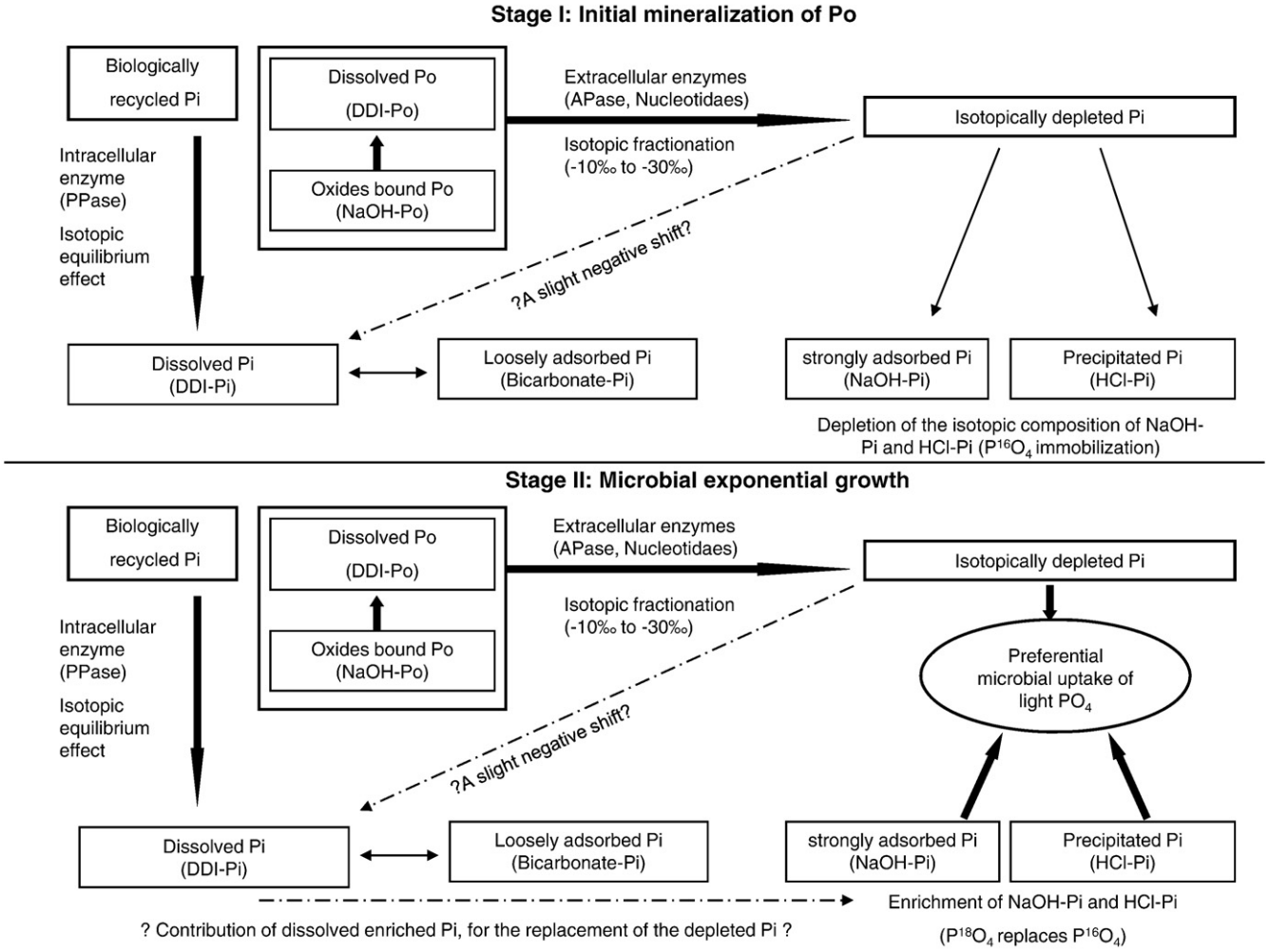

Fig. 6. The unifying model: the biological growth stages as affecting $\delta^{18}O_p$ of the soil Pi pools (thin lined boxes). Thick arrows represent biological processes; thin arrows represent geochemical processes; scattered arrows represent low impact potential effects.

by the rapidly growing cells, as was suggested by Blake and co-workers (2005), for *E. coli*. Thus, the isotopically depleted Pi pool which is sequestered by the oxides and minerals, during the first three days of incubation, may be biologically available to particle attached microorganisms and an important contributor to support the growth needs during the exponential growth phase, which likely occurred between day 3 and day 14 of incubation. Fig. 2 indicates that Po degradation continues in this time frame and we expect the resulting depleted Pi to be directly and preferentially assimilated by the growing biomass along with isotopically depleted Pi, from the adsorbed and precipitated pools.

For the adsorbed and precipitated $P^{16}O_4$ to be utilized by the soil microbes, isotope or molecular exchange must take place. Isotopic exchange, which involves replacement of one (or more) oxygen in PO_4 by another, was suggested previously to explain anomalous isotope values (McArthur and Herczeg, 1990; Ayliffe et al., 1992). The P–O bond is very strong and its breakage requires enzymatic catalyzation or high temperatures (>50 °C, Lecuyer et al., 1999), not maintained in our experiment. We are not aware of extracellular enzymatic activity which can result in Pi isotopic enrichment, other than activity of expelled PPase (when equilibrium value is higher than the initial value as in our experiment). Another option to explain the trends seen is molecular exchange, where replacement of depleted phosphate molecules by enriched phosphate molecules takes place via simultaneous processes of desorption–adsorption of Pi from Fe/Al soil oxides surfaces and dissolution–precipitation of P minerals. Fixation of Pi by oxides and mineral phases (i.e. NaOH–P and HCl–P fractions) is considered relatively strong, and the respective P is not readily bio-available (Tiessen and Moir, 1993). However, attached microorganisms cells, largely fungi and bacteria, may induce mineral and Fe-oxide dissolution by acid production and by excretion of organic ligands and high molecular weight polymers (Banfield et al., 1999 and references therein). Po mineralization in this study may have caused acidic microenvironments on soil surfaces. In addition, metastable P minerals (i.e., relatively easily dissolved minerals, such as brushite) are expected to be the first to precipitate shortly after P application to soil (Lindsay, 1979) and their persistence in the soil is enhanced by presence of humic and fulvic acids (Inskeep and Silvertooth, 1988; Grossl and Inskeep, 1991; Delgado et al., 2002), which can be found in RWW (Imai et al., 2002; Ilani et al., 2005). These desorption/adsorption and dissolution/precipitation processes may result in the release of previously bound Pi with low $\delta^{18}O_p$ to solution and its replacement by heavier Pi, e.g., Pi from solution, which carries the equilibrium signature. Further research is needed to determine conclusively which mechanism(s) take place in the isotopic transformations seen for the oxide and mineral associated Pi fractions.

Enrichment of the NaOH–$\delta^{18}O_p$ and HCl–$\delta^{18}O_p$ of the RWW soil during the first 14 days progressed similarly and in a parallel manner (~2‰ constant difference between the curves), while the FWF soil's HCl–$\delta^{18}O_p$ enrichment was less rapid (Figs. 2 a,b and 5). Since we assume the isotopic enrichment is associated with the exponential growth phase of soil microbes, the difference in slope between the two soil samples (Fig. 5) may be the result of different microbial communities, which operate at different rates, because of the experimental and historical different treatments of the two soils (wastewater vs. fertilizer). Indeed, it has been reported that microbial activity is higher in soils treated with biosolids, than in the same type of soils, treated with chemical fertilizer (Crouse et al., 2002; Oehl et al., 2004). Different rates of biological activity may therefore explain the different rates of enrichment.

Analysis of the original soil samples yielded low $\delta^{18}O_p$ for these soil fractions (Table 4), which following the current study's findings indicate that the oxides and P minerals extracted by NaOH and HCl solutions, in active soils like agricultural soils, do not retain the original $\delta^{18}O_p$ of the parent rock. Instead, irrigation several days before sampling has likely initiated biological growth, which induced Po mineralization yielding depleted Pi, which was sequestered by the oxides and P minerals and thus resulted in low $\delta^{18}O_p$ of those P fractions in the original RWW and FWF soil samples.

4.4. A unifying model

A unifying model for P transformations in soils based on our results from the RWW soil sample can be constructed. Fig. 6 presents the principle biogeochemical processes in this soil that may impact P cycling and transformations. As was mentioned before, we expect very similar processes to take place in the FWF treated soil, but the higher biological activity in the RWW treated soil enables clearer demonstration of the biogeochemical processes observed.

RWW application, at the beginning of the experiment, has induced microbial growth which progressed through a lag phase, exponential growth and a sustainability phase. Different microbial populations in the soil had different effects on soil P:

Following P application, the soil microbial populations become more active and extensive intracellular recycling of Pi (involving PPase activity), primarily by the microbial population in the soil solution, takes place and continues throughout the incubation month. Intracellular recycled Pi is expelled and released to solution, imprinting an enzymatic equilibrium signature within the more labile P pool (DDI–Pi). This equilibrium process overprints previous mixing or other signatures of P in solution and also interacts and impacts the loosely adsorbed Pi fraction (bicarbonate–Pi).

As microbial populations grow, they require nutrition resources. Soil Pi is plentiful, but C and N may limit growth. In the preliminary lag phase, starting immediately after the 'irrigation event', OM is mineralized by extracellular enzymes (APase and 5′-nucleotidase) to supply these nutritional requirements. This mineralization converts Po in soil solution (DDI–Po) and Po adsorbed by oxides (NaOH–Po) to Pi, a process that involves oxygen isotopic fractionation (− 10‰ to − 30‰), resulting in an isotopically depleted Pi pool. Pi from this isotopically depleted pool is immediately sequestered by oxides (NaOH–P) and by P minerals (HCl–P). Microbial communities that are associated with soil surfaces (possibly different populations than solution populations) are likely responsible for the enzymatic activity that directly impacts soil oxides and P minerals.

As the microbial community undergoes exponential growth the requirement for Pi increases; isotopically depleted Pi is preferentially taken up. Sources for this Pi uptake are continuous Po degradation and the vast amount of Pi sorbed by oxides and by P minerals. Attached cells induce on-site replacement of light Pi by heavy Pi, via destabilization of oxides and minerals. The source of heavy Pi for this exchange process likely originates in the dissolved Pi pool, which carries a heavier isotopic signature. This exchange results in an increase in the $\delta^{18}O_p$ of the NaOH and HCl Pi pools over time. During this exponential growth phase (first two weeks of incubation) Po is continuously remineralized, affecting the dissolved Pi pool and causing a slight negative shift from equilibrium values. OM degradation may have also resulted in increase in adsorption sites on clay minerals, which rapidly get reoccupied by Pi (bicarbonate–Pi).

After this period, during the sustainability phase, when the soil's microbial activity is reduced, sorbed P becomes more stable. Therefore, dissolved Pi does not affect sorbed Pi anymore and NaOH and HCl Pi pools retain the isotopic composition they last obtained at the end of the exponential growth.

5. Conclusions

Tracking changes in $\delta^{18}O_p$, in association with P concentration changes, was found to be a valuable complementary technique for the study of P transformations in the soil. The isotopic data reveal P dynamics which have not previously been appreciated using

concentrations alone. In the current study we demonstrate for the first time, that the soil P fractions which are characterized by strong binding by oxides and minerals (i.e., the NaOH and the HCl extracted P), and are considered as non labile, are affected by biological cycling of P by microbial populations, which probably inhabit oxide and mineral surfaces in the soil. The changing $\delta^{18}O_p$ of those P fractions may be related to microbial growth phases. The most labile P pool (DDI fraction) reflects primarily equilibrium isotope conditions, which results from intracellular recycling of Pi, by microbial populations in the soil solution, likely decoupled from populations on soil surfaces. The relatively labile P pool (bicarbonate extract) reflects continues communication and exchange with the dissolved soil Pi pool. These soil microbial processes, that manifest themselves in the isotopic signature of the tested soil fractions, enable estimation of relative rates of reactivity, when tracked over time. The organic phosphate in the soil solution and the strongly adsorbed Po (NaOH–Po) appear to have a major role in P transformations, impacting all soil P fractions. However, Po compounds are also utilized as a source of C and N indicating that cycling of P is strongly related to the availability and cycles of those constituents. Both soil samples, regardless of irrigation water type or fertilization history, appear to go through very similar biogeochemical processes, though at different rates. The RWW treated soil, due to higher availability of nutrients, was more reactive than the FWF treated soil. However, while fast Pi recycling sometimes reduces labile Pi concentrations, the RWW soil sample maintained higher concentrations of labile Pi, throughout the incubation month. The fact that the FWF soil sample had also retained high concentration of labile P, following the 'irrigation event' and throughout the incubation month, implies that application of excess P might be more important in determining P availability than the different historical irrigation treatments and related soil characteristics in certain cases.

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References

Ammerman, J.W., Azam, F., 1991. Bacterial 5′-nucleotidase activity in estuarine and coastal marine waters: role in phosphorus regeneration. Limnol. Oceanogr. 36, 1437–1447.

Ayliffe, L.K., Chivas, A.R., 1990. Oxygen isotope composition of the bone phosphate of Australian kangaroos: potential as a paleoenvironmental indicator. Geochim. Cosmochim. Acta 54, 2603–2609.

Ayliffe, L.K., Veeh, H.H., Chivas, A.R., 1992. Oxygen isotopes of phosphate and the origin of island apatite deposits. Earth Planet. Sci. Lett. 108, 119–129.

Bakker, C., Rodenburg, J., Bodegom, P., 2005. Effects of Ca- and Fe-rich seepage on P availability and plant performance in calcareous dune soils. Plant Soil 275, 111–122.

Banfield, J.F., Barker, W.W., Welch, S.A., Taunton, A., 1999. Biological impact on mineral dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. PNAS 96, 3404–3411.

Barrow, N.J., 1972. Influence of solution concentration of calcium on the adsorption of phosphate, sulfate, and molybdate by soils. Soil Sci. 113, 175–180.

Blagodatsky, S.A., Heinemeyer, O., Richter, J., 2000. Estimating the active and total soil microbial biomass by kinetic respiration analysis. Biol. Fertil. Soils 32, 73–81.

Blake, R., O'neil, J.R., Garcia, G.A., 1997. Oxygen isotope systematics of biologically mediated reactions of phosphate: I. Microbial degradation of organophosphorus compounds. Geochim. Cosmochim. Acta 61, 4411–4422.

Blake, R., O'Neil, J.R., Surkov, A.V., 2005. Biogeochemical cycling of phosphorus: insights from oxygen isotope effects of phosphoenzymes. Am. J. Sci. 305, 596–620.

Carter, M.R., 1996. Analysis of soil organic matter storage in agroecosystems. In: Carter, M.R. and Stewart, B.A. (Editors). Structure and organic matter storage in agricultural soils. CRC press, Inc. (Lewis press) Boca Raton, Fa.

Chrost, R.J., 1991. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: Chrost, R.J. (Ed.), Microbial enzymes in aquatic environments. Springer-Verlag, pp. 29–59.

Colman, A.S., Blake, R.E., Karl, D.M., Fogel, M.L., Turekian, K.K., 2005. Marine phosphate oxygen isotopes and organic matter remineralization in the oceans. PNAS 102, 13023–13028.

Criss, R.E., 1999. Principles of stable isotope distribution. Oxford University Press, US.

Crouse, D.A., Sierzputowska-Gracz, H., Mikkelsen, R.L., Wollum, A.G., 2002. Monitoring phosphorus mineralization from poultry manure using phosphatase assays and ^{31}P-NMR spectroscopy. Commu.Soil Sci. Plant Anal. 33, 1205–1217.

Delgado, A., Madrid, A., Kassem, S., Andreu, L., del Carmen del Campillo, M., 2002. Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. Plant Soil 245, 277–286.

Elsbury, K.M., Paytan, A., Ostrom, N.E., Kendall, C., Young, M.B., McLaughlin, K., Watson, S., 2009. Using oxygen isotopes of phosphate to trace phosphorus sources and cycling in lake Erie. Environ. Sci. Technol. 43, 3108–3114.

Falkiner, R.A., Polglase, P.J., 1999. Fate of applied phosphorus in an effluent-irrigated Pinus radiate plantation. Aust. J. Soil Res. 37, 1095–1106.

Fricke, H.C., Clyde, W.C., O'Neil, J.R., Gingerich, P.D., 1998. Evidence for rapid climate change in North America during the latest Paleocene thermal maximum: oxygen isotope compositions of biogenic phosphate from the Bighorn Basin (Wyoming). Earth Planet. Sci. Lett. 160, 193–208.

Grossl, P.R., Inskeep, W.P., 1991. Precipitatrion of dicalcium phosphate dehydrate in the presence of organic acids. Soil Sci. Am. J. 55, 670–675.

Guggenberger, G., Christensen, B.T., Rubaek, G.H., 2000. Isolation and characterization of labile organic phosphorus pools in soils from the Askov long term field experiments. J. Plant Nutr. Soil Sci. 163, 151–155.

Hansen, J.C., Cade-Menun, B.J., Strawn, D.G., 2004. Phosphorus speciation in manure amended alkaline soils. J. Environ. Qual. 33, 1521–1527.

Hedley, M.J., Stewart, J.W.B., Chauhan, B.S., 1982. Changes in inorganic and organic soil phosphorus fractions induced by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970–976.

Ilani, T., Schulz, E., Chefetz, B., 2005. Interactions of organic compounds with wastewater dissolved organic matter: role of hydrophobic fractions. J. Environ. Qual. 34, 552–562.

Imai, A., Fukushima, T., Matsushige, K., Kim, Y.H., Choi, K., 2002. Characterization of dissolved organic matter in effluents from wastewater treatment plants. Water Res. 36, 859–870.

Inskeep, W.P., Silvertooth, J.C., 1988. Inhibition of hydroxyapatite precipitation in the presence of fulvic, humic and tannic acids. Soil Sci. Soc. Am. J. 52, 941–946.

Jaisi, D.P., Blake, R.E., Kukkadapu, R.K., 2010. Fractionation of oxygen isotopes in phosphate during its interactions with iron oxides. Geochim. Cosmochim. Acta 74, 1309–1319.

Khasawneh, F.E., Sample, E.C., Kamprath, E.J. (Eds.), 1980. The Role of Phosphorus in Agriculture. Am. Soc. Agron. Madison, Wis.

Kolodny, Y., Luz, B., Navon, O., 1983. Oxygen isotope variations in phosphate of biogenic apatites, I. Fish bone apatite—rechecking the rules of the game. Earth Planet. Sci. Lett. 64, 398–404.

Larsen, S., Middelboe, V., Saaby, J.H., 1989. The fate of oxygen-18 labeled phosphate in soil/plant systems. Plant Soil 117, 143–145.

Lecuyer, C., Grandjean, P., Sheppard, S.M.F., 1999. Oxygen isotope exchange between dissolved phosphate and water at temperatures $\leq$135 °C: inorganic versus biological fractionations. Geochim. Cosmochim. Acta 63, 855–862.

Liang, Y., Blake, R.E., 2006. Oxygen isotope signature of P_i regeneration from organic compounds by phosphomonoesterases and photooxidation. Geochim. Cosmochim. Acta 70, 3957–3969.

Lindsay, W.L., 1979. Phosphates. Chemical equilibria in soils. A Wiley Interscience publication, pp. 162–209.

Lis, G., Wassenaar, L.I., Hendry, M.J., 2008. High precision laser spectroscopy D/H and $^{18}O/^{16}O$ measurements of microliter natural water samples. Anal. Chem. 80, 287–293.

Longinelli, A., 1989. Oxygen-18 and Sulphur-34 in Dissolved Oceanic Sulphate and Phosphate. In: Fritz, P., Fontes, J.Ch. (Eds.), Handbook of Environmental Isotope Geochemistry. Elsevier, Amsterdam, pp. 219–255.

Longinelli, A., Nuti, S., 1973. Oxygen isotope measurements of phosphate from fish teeth and bones. Earth Planet. Sci. Lett. 19, 373–376.

Longinelli, A., Bartelloni, M., Cortecci, G., 1976. The isotopic cycle of oceanic phosphate, I. Earth Planet. Sci. Lett. 32, 389–392.

Markel, D., Kolodny, Y., Luz, B., Nishri, A., 1994. Phosphorus cycling and phosphorus sources in lake Kinneret: tracing by oxygen isotopes in phosphate. Isr. J. Earth Sci. 43, 165–178.

McArthur, J.M., Herczeg, A., 1990. Diagenetic Stability of the Isotopic Composition of Phosphate–Oxygen: Palaeoenvironmental Implications. In: Notholt, A.J.G., Jarvis, I. (Eds.), Phosphorite research and development, vol. 52. Geol. Soc. London Special Pub., London, pp. 119–124.

McLaughlin, K., Silva, S., Kendall, C., Stuart-Williams, H., Paytan, A., 2004. A precise method for the analysis of $\delta^{18}O$ of dissolve inorganic phosphate in seawater. Limnol. Oceanogr. Meth. 2, 202–212.

McLaughlin, K., Cade-Menun, B.J., Paytan, A., 2006a. The oxygen isotopic composition of phosphate in Elkhorn Slough, California: a tracer for phosphate sources. Estuar. Coast. Mar. Sci. 70, 499–506.

McLaughlin, K., Kendall, C., Silva, S.R., Young, M., Paytan, A., 2006b. Phosphate oxygen isotope ratios as a tracer for sources and cycling of phosphate in North San Francisco Bay, California. J. Geophys. Res. 111, G03003.

Middelboe, V., Saaby Johansen, H., 1998. Quantification of cumulative bioactivity in soil via replacement of oxygen in labeled phosphate. Appl. Radiat. Isot. 49, 855–857.
Morgan, M.A., 1997. The Behaviour of Soils and Fertilizer Phosphorus. In: Tunney, H., Carton, O.T., Brooks, P.C., Johnston, A.E. (Eds.), Phosphorus loss from soil to water. CAB INTERNATIONAL, Wallingford, pp. 137–149.
Oehl, F., Frossard, E., Fliessbach, A., Dubois, D., Oberson, A., 2004. Basal organic phosphorus mineralization in soils under different farming systems. Soil Biol. Biochem. 36, 667–675.
Paytan, A., Kolodny, Y., Neori, A., Luz, B., 2002. Rapid biologically mediated oxygen isotope exchange between water and phosphate. Glob. Biogeochem. Cycles 16, 1–7.
Saaby Johansen, H., Larsen, S., Middleboe, V., 1989. The stability of labeled phosphate in aqueous solutions. Appl. Radiat. Isot. 40, 641.
Shemesh, A., Kolodny, Y., Luz, B., 1983. Oxygen isotope variations in phosphate of biogenic apatites, II. Phosphorite rocks. Earth Planet. Sci. Lett. 64, 405–416.
Sibbesen, E., Sharpley, A.N., 1997. Setting and Justifying Upper Critical Limits for Phosphorus in Soils. In: Tunney, H., Carton, O.T., Brooks, P.C., Johnston, A.E. (Eds.), Phosphorus loss from soil to water. CAB INTERNATIONAL, Wallingford, pp. 151–176.
Stroud, J.L., Paton, G.I., Semple, K.T., 2007. Microbe-aliphatic hydrocarbon interactions in soil: implications for biodegradation and bioremediation. J. Appl. Microbiol. 102, 1239–1253.
Sui, Y., Thompson, M.L., Shang, C., 1999. Fractionation of phosphorus in a mollisol with biosolids. Soil Sci. Soc. Am. J. 63, 1174–1180.
Taranto, M.T., Adams, M.A., Polglase, P.J., 2000. Sequential fractionation and characterization (^{31}P-NMR) of phosphorus-amended soils in Banksia integrifolia (L.f.) woodland and adjacent pasture. Soil Boil. Biochem. 32, 169–177.
Tarchitzky, J., 2004. Israel National Survey of Effluent Effects, Ministry of Agriculture, The state of Israel. (in Hebrew).
Tiessen, H., Moir, J.O., 1993. Characterization of Available P by Sequential Extraction. In: Carter, M.R. (Ed.), Soil sampling and methods of analysis. Lewis, pp. 75–86.
Tunney, H., Carton, O.T., Brooks, P.C., Johnston, A.E. (Eds.), 1997. Phosphorus loss from soil to water. CAB INTERNATIONAL, New York.
Zheng, Yong-Fei, 1996. Oxygen isotope fractionations involving apatites: application to paleotemperature determination. Chem. Geol. 127, 177–187.
Zohar, I., Shaviv A., Klass T., Roberts K. and Paytan A., submitted. A method for the analysis of oxygen isotopic composition of various soil phosphate fractions. Environ. Sci. Tech.