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Oxygen Isotopes Unravel the Role of Microorganisms in Phosphate Cycling in Soils

Federica Tamburini,*[,†] Verena Pfahler,[†] Else K. Bünemann,[†] Kathi Guelland,[‡] Stefano M. Bernasconi,[§] and Emmanuel Frossard[†]

[†]Institute of Agricultural Sciences, ETH Zurich, 8092 Zurich, Switzerland

[‡]Waldböden und Biochemie, WSL, 8903 Birmensdorf, Switzerland

[§]Geological Institute, ETH Zurich, 8092 Zurich, Switzerland

Supporting Information

ABSTRACT: Phosphorus (P) is considered the ultimate limiting nutrient for plants in most natural systems and changes in the distribution of inorganic and organic P forms during soil development have been well documented. In particular, microbial activity has been shown to be an important control on P cycling but its contribution in building up the pool of plant-available P during soil development is still poorly quantified. To determine the importance of different biological processes on P cycling, we analyzed the isotopic composition of oxygen in phosphate (δ^{18}O-Pi) from the parent material, soil microorganisms, the available P pool, and from the vegetation along a 150-year soil chronosequence of a glacier forefield. Our results show that at all sites, δ^{18}O-Pi of microbial Pi is within the range expected for the temperature-dependent equilibrium between phosphate and water. In addition, the isotopic signature of available Pi is close to the signature of microbial Pi, independently of the contribution of parent material Pi, vegetation Pi or Pi released from organic matter mineralization. Thus, we show that phosphate is cycled through soil microorganisms before being released to the available pool. This isotopic approach demonstrates for the first time in the field and over long time scales, and not only through controlled experiments, the role of the microbial activity in cycling of P in soils.

INTRODUCTION

Phosphorus is an essential element for life[1] as it is present in biological molecules such as DNA and RNA, and it participates in many metabolic reactions. Whereas the effects of sorption and desorption on minerals in controlling inorganic orthophosphate (Pi) availability in soils are rather well understood,[2] still little is known about the importance of biological processes. In general, during soil development, available Pi concentration first increases due to weathering and then decreases as Pi becomes sequestered in organic forms, occluded in secondary minerals, and lost from the soil profile.[3,4] In mature soils, biological processes such as the production of extracellular enzymes by plant roots and microorganisms induce the release of Pi, counteracting P limitation.[4] After having taken up Pi, the microbial biomass can release it, becoming a source of Pi.[5] This release can be accelerated by microbial grazing by the micro- and macrofauna[6] and by strong fluctuations in weather conditions such as wetting/drying events.[7]

Analyzing and quantifying the P content in different soil pools provide limited information on the processes phosphate went through. Together with plant uptake, phosphate mobilization and mineralization by soil microbes are among the most important biological processes impacting soil P availability.[8] Although incubation studies have shown the impact of microbial activity on P transformations,[5,9,10] there is no direct evidence of the role of microbial activity on P immobilization and release from field measurements.[10] One of the reasons is that P has only one stable isotope (^{31}P), and that the two radioactive isotopes (^{32}P and ^{33}P) can only be used in short-term studies.[11] Biological processes have been shown to alter the isotopic composition of oxygen associated with P in orthophosphate (δ^{18}O-Pi),[12] whereas oxygen isotope exchange with water does not occur at earth-surface temperatures without biological mediation. Biological uptake and cell-internal Pi cycling, together with the action of extracellular enzymes, are the processes with the largest impact on δ^{18}O-Pi.[13,14] Therefore, determining the oxygen isotope composition of phosphate is a powerful tool to study the biological cycling of P.

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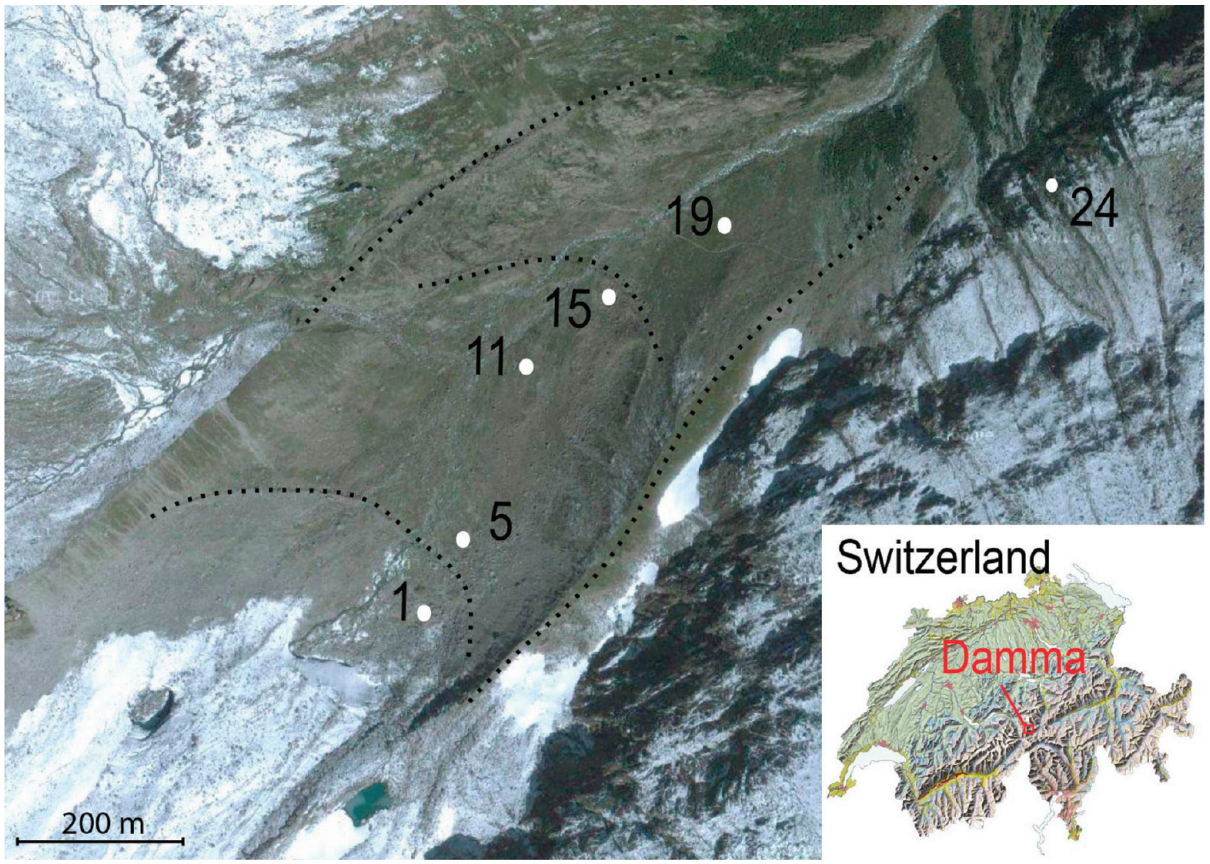

Figure 1. Damma glacier forefield map and site locations. Dotted lines represent the position of frontal and lateral moraines.

Previous studies of δ^{18}O-Pi in terrestrial environments focused on understanding processes in short- term incubation or field experiments,[15−17] or on tracing P sources from soils to water bodies.[18] Forefields of retreating glaciers are natural laboratories for studying soil formation processes, since their soils share the same parent material and climate but show different degrees of biological activity and soil development. The objectives of our study were to measure the δ^{18}O of different Pi pools in soil samples to identify the biological processes controlling P availability to plants and microbes during early stages of soil development.

MATERIALS AND METHODS

Field Description and Sample Collection. We collected samples from the 150-year old Damma glacier soil chronosequence (Figure 1), which has been extensively studied in the framework of the multidisciplinary project BigLink.[19] The Damma glacier forefield is located in the Central Swiss Alps between approximately 1900 and 2100 m above sea level, and it is covered by snow for about 8 months each year. Average rain and snow precipitation is about 2400 mm per year. Bedrock is constituted of metamorphosed granite, with apatite as accessory mineral.[19] Soil organic carbon increases and pH decreases along the chronosequence.[19] The vegetation evolves from few grasses in the young sites to full coverage with *Leucanthemopsis alpina*, *Agrostis alpina* and *gigantea*, *Salix herbacea* and *helvetica*, *Rhododendron ferrugineum* at the older sites.[20]

In July 2010, we choose five of the BigLink common soil sampling sites, with ages ranging between 10 and 120 years, plus a reference site at least 3000 years old located outside the chronosequence (site 24, Figure 1). Soil age was calculated using the distance from the glacier and measured rates of glacier retreat.[19] At each site, we took a representative sample of the 0−5 cm horizon by sampling at least in three different spots about 1 kg of soil, which was then homogenized. Despite the relative vicinity of the sampled sites to each other, sites 1 and 11 tend to be often wet, due to the proximity to ice (site 1) and to the stream (site 11), while the other sites (5, 15, and 19) are drier and more vegetated. Soil temperatures were recorded in July 2008 by using temperature loggers (iButton, Maxim, Sunnyvale, CA, USA), which were buried at each site at approximately 2.5 cm depth. Temperature were recorded at 1-h interval and then averaged, with a precision of 0.5 °C. Soil−water was sampled in July 2008 using glass suction plates (⌀ 5.5 cm made of borosilicate glass, porosity P5, corresponding to a pore size of 1−1.6 μm), which were installed at a soil depth of 10 cm and connected to 250 mL glass bottles. A suction of approximately 400hPa was applied manually with a 100 mL syringe to collect soil−water. Prior to installation, the glass plates were flushed with 1 M HCl and cleaned with deionized water. To minimize biological activity, the soil−water samples were continuously cooled. The sampled soil−water was transported to the laboratory, where it was filtered through 0.45 μm cellulose-acetate filters within 48 h of sampling. We assume that δ^{18}O values of soil−water sampled in July 2008, together with soil temperatures measured in the same period, show average values and variability that can be expected in the forefield in the month of July.

Phosphorus Characterization and Phosphatase Activity Measurements. The soil samples were sieved at <2 mm in the field and homogeneized. Phosphate was measured from five different soil pools obtained in three different extractions (i.e.,

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Table 1. Stocks of Pi and Po (in mg or g of Phosphorus Per Square Meter) for the Top 5 cm of Damma Soils[a]

site	vegetation Pi (mg P/m^2)	available Pi (mg P/m^2)	microbial Pi (mg P/m^2)	oxides-Pi (g P/m^2)	Po (g P/m^2)	mineral Pi (g P/m^2)	Pase activity (mg P/kg/h)
1	0.22	19.1	37.4	0.21	0.28	18.9	0.79
5	19.3	28.9	56.5	0.30	0.22	27.4	1.48
11	25.4	22.7	168	0.48	1.36	22.6	16.4
15	44.0	52.1	725	0.95	1.99	23.9	62.2
19	52.8	41.4	699	1.11	3.36	10.3	75.1
24	49.3	11.6	286	6.48	50.2	9.61	259

[a]Phosphomonoesterase (Pase, mainly acid phosphatase) activity is expressed in mg of P per kg of soil per hour.

we did not carry out a sequential extraction). Resin extractable Pi represents Pi from the soil solution and Pi that is loosely sorbed on soil particles (noted thereafter as available Pi). Microbial Pi is defined as the amount of P extracted from the soil by the resin in the presence of hexanol minus the amount of P extracted from the soil without hexanol. For microbial and resin Pi extractions, 20 g of fresh soil was used, to minimize the impact of heterogeneity and obtain enough Pi for isotopic analysis. Microbial Pi and soil available Pi were extracted at 4 °C by using anion resin membranes (BDH-551642S available from VWR) saturated with HCO_3^{2-} in a soil:water suspension, and in the presence and absence of hexanol.[21] The use of fresh soil for these analyses is fundamental, since it is proven that Pi extracted by resin from dried soil does not only represent available Pi, but also part of microbial Pi.[7] Soil quantities, soil:solution ratio and the number of resin membranes used for these extractions were scaled up to obtain enough P for isotope analysis. Extracted phosphate was then eluted from the membranes with 0.2 M HNO_3. In a distinct step, we extracted soil samples with 1 M HCl using about 10 g of soil previously dried at 40 °C.[22] HCl extractable Pi (noted thereafter as mineral Pi) is considered as a good approximation of Pi present in Ca−P minerals such as apatite. Soils were also extracted with NaOH−EDTA, which solubilizes Pi sorbed to Al and Fe oxides (noted thereafter as oxides-Pi) and organic P forms (noted thereafter as Po). This extraction was performed on 3 g of dried soil with 30 mL of a 0.25 M NaOH−0.05 M EDTA solution. The difference between total P in the solution and oxides-Pi gives an estimate of the soil organic P content (Po). Plant Pi was extracted from fresh leaves with 0.3 M trichloroacetic acid (TCA)[23] at 4 °C from about 2 g of fresh vegetation material. Phosphate concentrations in solution were determined using the malachite green colorimetric technique.[24] The concentrations of total P in NaOH−EDTA extracts were measured colorimetrically after acid persulfate digestion at 110 °C.[25] Phosphorus stocks (Table 1) are reported in mg (or g) of P per square meter down to a depth of 5 cm, by using a soil density value of 8.9 kg/m^2/cm depth.[26] For vegetation P stocks, we used vegetation cover estimates.[19]

For phosphatase activity measurements, soil was sampled separately. After been sieved at 8 mm in the field, it was transported to the lab, sieved moist at <2 mm and frozen immediately at −20 °C. Phosphomonoesterase activity was measured in a microplate design.[27] One gram of frozen soil was dispersed in 100 mL of autoclaved H_2O using an ultrasonic probe. The suspension was transferred to a microplate with three analytical replicates, 4-methylumbelliferyl-phosphate was used as substrate, and the assay was buffered with 0.1 M MES buffer at pH 6.1. The increase in fluorescence was measured over 180 min (FLx800, Biotek). At pH 6.1, the vast majority of the measured activity can be attributed to acid rather than to alkaline phosphatases.[28]

Sample Preparation for Isotope Analysis and Measurements. Phosphate extracted from the available, microbial, and mineral Pi pools and vegetation was purified and converted to silver phosphate.[22] ^{18}O-labeled and unlabeled extraction solutions (for TCA and HCl extractions) were used to verify the presence of condensed and organic P compounds in HCl Pi and vegetation.[22] When the amount of phosphate in the extracted solution was lower than 10 micromoles (as in resin Pi extracts), a modified ammonium-phosphomolybdate precipitation was used.[29] The δ^{18}O of microbial Pi was calculated by mass balance, using concentration values of resin-hexanol Pi and resin Pi and their isotopic signatures. Oxygen isotope analysis was carried out with a high temperature conversion elemental analyzer (Thermo Scientific TC/EA), coupled in a continuous flow to an isotope ratio mass spectrometer (Thermo Scientific Delta V plus). All values are reported in the conventional per mil notation (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW).

We decided not to attempt the purification of Pi and total P extracted by NaOH−EDTA for isotope analysis because (i) oxides-Pi in soils is in a dynamic exchange with the soil available Pi,[11] so although it constitutes an important pool in term of size, it retains the isotopic signature of the soil available Pi[13,17] and (ii) because we do not have yet a protocol for purifying P from the organic phase of the NaOH−EDTA extract although the δ^{18}O of Po would be of great interest.[14]

Oxygen isotopes in soil−water were measured by equilibration with CO_2.[30] Two hundred microliters of water was pipetted in 12 mL septum-capped vials, which were subsequently filled with a mixture of 0.3% CO_2 and He. After equilibration at 25 °C for at least 18 h, the CO_2/He mixture was measured using a gas bench (Thermo Scientific Gas Bench II) connected to an isotope ratio mass spectrometer (Thermo Scientific Delta V plus). The system was calibrated with the international standards SMOW (Standard Mean Ocean Water), SLAP (Standard Light Antarctic Precipitation), and GISP (Greenland Ice Sheet Precipitation), distributed by the International Atomic Energy Agency, Vienna, Austria. The results are reported in the conventional delta notation with respect to VSMOW. Reproducibility of the measurements based on repeated measurements of an internal standard was better than 0.06‰.

RESULTS AND DISCUSSION

Mineral Pi decreased by 65%, from the younger to the older sites while oxides Pi and Po increased (Table 1). Stocks of available Pi were low, with the highest value observed in the intermediate-age soils. The microbial Pi stock increased in sites older than 60 years and plant Pi stocks were comparable to

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available Pi stocks. Extracellular acid phosphatase activity was low at the youngest sites, but increased strongly with soil age reaching a maximum at site 24. The $\delta^{18}O$ of mineral Pi extracted by ^{18}O-labeled and unlabeled HCl indicated that organic or condensed phosphates such as poly- or pyrophosphates were not present in this extract. The $\delta^{18}O$ of mineral Pi was constant (about +7‰), while only at site 24 mineral Pi showed a heavier $\delta^{18}O$. *Agrostis* foliage averaged +22‰, whereas the isotopic signature of the other plants varied between +4.5 and +31‰ (Supporting Information, Table S1), with a weighted average of +20.4‰. Microbial and available Pi pools showed values ranging between +11 and +19‰ (Figure 2).

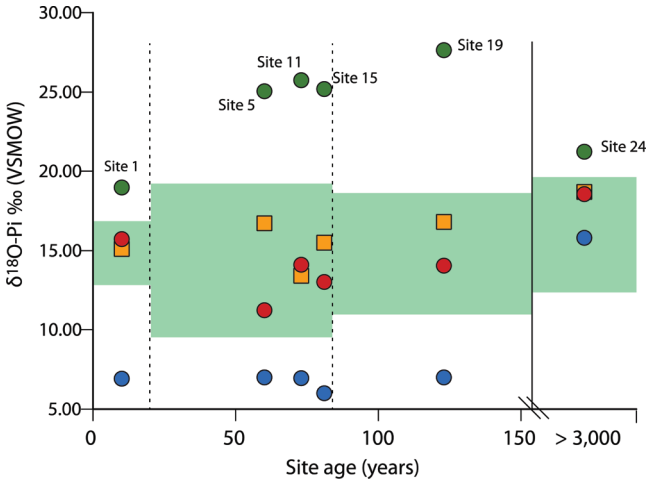

Figure 2. Stable oxygen isotopes bound in phosphate extracted from three soil pools and from vegetation. Blue, green and red dots represent $\delta^{18}O$-Pi values of mineral Pi, vegetation and microbial Pi pools, respectively, while yellow squares represent $\delta^{18}O$-Pi values of available Pi. The two dotted lines represent the age of the frontal moraines. The green shaded area represents the equilibrium window calculated using ranges of soil temperatures and oxygen isotope values of soil–water as recorded for the month of July (Table S2).

Among the biological processes controlling the $\delta^{18}O$ of Pi, the activity of inorganic pyrophosphatase (PPase) appears to be the most influential.[13] PPase is essential in many intracellular metabolic processes as it controls the hydrolysis of inorganic pyrophosphate to orthophosphate. Both the hydrolysis of pyrophosphate and the formation of an enzyme-orthophosphate intermediate lead to a complete equilibration of the oxygen isotopes between phosphate and water[31] with a temperature-dependent fractionation, which decreases by 0.23‰ per °C temperature increase.[32] We could thus define an equilibrium window between oxygen in phosphate and water for all sites (green shaded area in Figure 2). The boundaries of this equilibrium window were computed by using the average daily soil temperatures and the $\delta^{18}O$ of soil–water for July (Supporting Information, Table S2) in the equation of Longinelli and Nuti (1973), which describes empirically the relation between phosphate, water and temperature.[32]

$$T(°C) = 111.4 - 4.3(\delta^{18}O - Pi - \delta^{18}O - H_2O)$$

where $\delta^{18}O$–Pi is the isotopic signature of oxygen in a phosphate pool, while T and $\delta^{18}O$–H_2O are the temperature in degree Celsius and the isotopic signature of soil–water, respectively. At same sites, the range of the equilibrium window is quite wide (up to 10‰), which is determined by the large fluctuations of $\delta^{18}O$ of soil–water during the sampling period (about 3 weeks), and influenced by spatial heterogeneity in the field. For these reasons, the equilibrium window should provide an indication of the possible values expected in a relatively long period of time, and not only give a snapshot of the soil at the time of sampling. Indeed, in cell-free essays, where amounts of pyrophosphatase enzyme were well above natural concentrations, equilibrium between phosphate and water promoted by intracellular cycling was reached only in one to two days, depending on temperature.[13] It is thus expected that in natural environment the equilibrium would be reached in longer periods.

Mineral and vegetation Pi fell outside the equilibrium window. At all sites except site 24, mineral $\delta^{18}O$-Pi was significantly lower than the other pools and reflected its origin as igneous and/or metamorphic apatite.[33] Heavy $\delta^{18}O$-Pi values for vegetation were most likely due to equilibration of phosphate, via PPase or other similar enzymatic processes,[31] with leaf water, which compared to soil–water is enriched in ^{18}O because of evapotranspiration.[34] Intracellular cycling and equilibration with soil–water through the action of PPase within soil microorganisms can explain why the $\delta^{18}O$ of the microbial and available Pi pools are within the equilibrium window (Figure 2). At site 24, the $\delta^{18}O$ of mineral Pi also fell within the equilibrium window. We suggest that at this site HCl extracted also Pi bound to oxides[35] and not only P from the parent material. Because the isotopic fractionation associated with the sorption of Pi on oxides is in the order of +1‰,[36] the sorbed Pi would have a signature similar to that of the available Pi. Indeed, we measured increased oxides-Pi contents at this site which paralleled increased contents in Fe(III)-hydroxides.[37]

There are two possible mechanisms to equilibrate the available Pi pool with soil–water. Equilibration could occur through extracellular PPase in the soil solution or through intracellular PPase within soil microorganisms. Although PPase has been observed in soils,[38] the activity of PPase is certainly much higher within microbial cells. Therefore, we propose that there is a continuous flux of P between the available Pi pool and the microbial pool, where intracellular PPase brings Pi to equilibrium, and from which Pi is released back to the available Pi pool.

At some Damma sites, $\delta^{18}O$ of available Pi was heavier than microbial Pi, which is assumed to be at equilibrium (Figure 2). Deviations of $\delta^{18}O$-Pi from equilibrium with water have been observed in natural systems and attributed to contributions from sources with an out-of-equilibrium signature[17,18] or to the action of extracellular enzymatic activity,[39−41] or to the imprint of biological uptake.[17] In soils, a delay between microbial intracellular equilibration with soil–water and release to the soil solution could also produce an out-of-equilibrium signature.

Using the calculated Pi stocks, we built a box-model to estimate Pi fluxes and contributions from the mineral, microbial and vegetation pools to the available Pi pool[42] (Table 2). For microbial Pi, we considered three possible turnover rates: 20 days (as observed for microbial N in another glacier forefield),[43] 70 days,[5] and 365 days. For plant Pi, we considered a turnover time of one year from P uptake by the plants to its release to the soil after plant decay. Fluxes of Pi from the mineral pool (0.21 mg P/m^2/day) were calculated by dividing the difference in concentration by the difference in age between sites 19 and 1. Using these data, we modeled the expected isotopic signature of the available Pi pool and compared it with

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Table 2. Box Model: Estimates of Pi Fluxes from the Mineral Pi, Microbial and Vegetation Pi Pools to the Build-up of the Available Pi Pool

	δ^{18}O-Pi			Pi fluxes			δ^{18}O−Pi		
site	mineral (‰)	vegetation (‰)	microbial (‰)	mineral (mg P/m^2/day)	vegetation (mg P/m^2/day)	microbial (mg P/m^2/day)	available Pi measured (‰)	available Pi calculated (‰)	Δ (meas − calcd)
1	6.9	19	15.7	0.21	0.001	1.87[a]	15.1	14.8	0.3
						0.53[b]		13.2	1.9
						0.31[c]		12.2	2.9
5	6.8	25.1	11.2	0.21	0.053	2.83	16.7	11.2	5.5
						0.81		11	5.7
						0.47		11	5.7
11	7.0	25.8	14.1	0.21	0.070	8.41	13.4	14	−0.6
						2.40		13.9	−0.5
						1.40		13.7	−0.3
15	6.0	25.2	13.0	0.21	0.120	36.27	15.5	13	2.5
						10.36		13	2.5
						6.04		13	2.5
19	7.1	27.6	14.0	0.21	0.145	34.93	16.8	14	2.8
						9.98		14.1	2.7
						5.82		14.1	2.7
24	15.1	21.2	17.8	0.21	0.135	14.28	18.7	17.8	0.9
						4.08		17.8	0.9
						2.38		17.8	0.9

[a]Fluxes of microbial Pi were calculated using turnover rate estimates of 20 days. [b]Fluxes of microbial Pi were calculated using turnover rate estimate of 70 days. [c]Fluxes of microbial Pi were calculated using turnover rate estimate of 365 days.

the measured one (Table 2). At sites 1, 19 and 24, the difference between measured and calculated δ^{18}O-Pi was between 0.3 and 0.9‰, while at the remaining sites the difference was greater. Except at site 1, changes in the turnover rate of microbial Pi caused insignificant variations in the calculated δ^{18}O−Pi of the available Pi pool. At all sites, the contribution of the microbial Pi to the build-up of the available Pi pool was the most preeminent.

Phosphatase activity increased notably at the older sites, as a response to a higher vegetation density (Table 1) and, thus, to a stronger P limitation. The activity of extracellular phosphatases is accompanied by changes in the δ^{18}O−Pi of the released phosphate.[13] Depending on the enzyme (monoesterases vs diesterases), 3 to 2 oxygen atoms of the released phosphate ion are inherited from the original organic compound, while 1 to 2 oxygen atoms are exchanged with water, accompanied by a kinetic fractionation with values between −10 and −30‰.[14] Although the isotopic effect of acid phosphatase, the main enzyme class active at the Damma forefield (Table 1), is not known and only few enzymes have been studied,[14] the Pi released by extracellular enzymes is generally lighter than the phosphate in the original compound. For instance, Pi released from organic mineralization of plant material from the Damma forefield, would be expected to range between +13 and −8 ‰, depending on the plant or litter considered, the active enzyme and the δ^{18}O of the water. At the Damma soils, the measured enzymatic activity and the amount of Po (Table 1) suggest that Pi released by organic matter mineralization have contributed to the buildup of available Pi, at least in the more developed sites. However, a clear isotopic imprint of these enzymes on the available Pi pool could not be detected, as the isotopic signature of the available Pi is close or higher than the microbial pool δ^{18}O−Pi.

Under the low-P conditions of these soils, uptake of Pi by microorganisms and plants could have partially overprinted the signature of the available Pi pool.[17] In fact, biological uptake of phosphate can discriminate against the heavy isotopes, leaving the remaining pool enriched in the ^{18}O.[13] At present, there is no supporting information on the effect of plant and microbial uptake on the remaining soil available Pi pool. However, if confirmed also for uptake by vegetation, the fractionation factor of about −3‰ calculated for *E. coli*[13] could explain the heavier δ^{18}O-Pi of available Pi observed at certain sites.

Depending on the release rate of Pi from the microbial to the available Pi pool, there could be a delay in the transfer of the isotopic signature. As discussed previously, the complete O exchange between Pi and H_2O promoted by PPase can be fast.[13] The δ^{18}O of soil−water collected in July 2008 varied by up to 6‰ in three weeks (Supporting Information, Table S2), which equals to a 6‰ variation of the δ^{18}O-Pi, given a constant temperature. Unless the release of Pi from the microbial biomass is as fast as these variations (note that the shortest turnover rate was 20 days[43]), there will be a temporal "lag" between the isotopic signatures of the microbial and of the available Pi.

The above discussion shows that regardless of the contribution of Pi from the mineral or vegetation pools, and from the activity of extracellular enzymes, microbial biomass is the main controller of the δ^{18}O−Pi of soil available Pi. Most likely as soon as Pi is released from apatite by dissolution or from organic compounds by enzymatic activity, it is taken up by microbial biomass and Pi is equilibrated with soil−water inside the cells. Upon microbial death and cell lysis, Pi is then released back to the soil solution with an equilibrium signature. In fact, despite the low stock of microbial Pi compared to the large stock of P present in the parent material, the isotopic signature of the mineral pool has disappeared from the available Pi already at the youngest site. Likewise, despite high extracellular phosphatase activity at the oldest sites, the isotopic signature of available Pi was still at or close to equilibrium, indicating a rapid microbial Pi turnover.[5,9,15]

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A recent study[44] indicated that microbial communities isolated from the Damma soils could grow on granitic sand as the sole source of P. After 8 days of incubation, the growth medium was still depleted in P, suggesting that the P released from the mineral pool was continuously recycled to sustain the growing microbial community. However, under field conditions, C and N limitation,[20] but also microbial grazing, for example, by amoeba,[19] and drying-rewetting or freezing-thawing cycles can accelerate Pi microbial turnover and the rate of transfer of Pi from the microbial biomass to the available pool. This could explain why P is rarely a limiting element for plants and microorganisms in the early stages of soil development albeit low stocks of available Pi. The rapid microbial Pi turnover (about 20 days) at the site 1 shown by our box model seems to suggest that at the youngest site also carbon is not limiting,[10] supporting results obtained by bacterial growth incubations[20] and bacterial community studies.[45]

Summarizing, our study shows that the use of δ^{18}O-Pi of soil pools can lead to a better understanding of the biological processes involved in P cycling, and can also give insights on nutrient limitation. Our study of the Damma chronosequence shows the fundamental role of the microbial biomass in controlling the isotopic composition of available soil Pi, supporting conclusions from other studies and environments.[17,41] Most importantly, it provides the first direct field observation that soil Pi is cycled through the microbial biomass in a continuous way.

■ ASSOCIATED CONTENT

Supporting Information

The δ^{18}O values of soil pore water and the full data set of concentrations and δ^{18}O-Pi from vegetation appear in Table S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Address: Eschikon 33, 8315 Lindau, Switzerland. Telephone: +41 52 354 9281. Fax: +41 52 354 9119. E-mail: federica.tamburini@usys.ethz.ch.

Notes

The authors declare no competing financial interest.

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