**δ18O as a tracer of PO43- losses from agricultural landscapes**

Naomi S. Wells1,2\*, Daren C. Gooddy3, Mustefa Yasin Reshid1, Peter J. Williams3, Andrew C. Smith4, Bradley D. Eyre1

1Centre for Coastal Biogeochemistry, School of Environment, Science & Engineering, Southern Cross University, PO Box 157, East Lismore, 2480 NSW, Australia

2Department of Soil & Physical Sciences, Faculty of Agricultural & Life Sciences, Lincoln University, Lincoln 7647, New Zealand

3British Geological Survey, Wallingford, Oxfordshire, OX10 8BB, UK

4British Geological Survey, Keyworth, Nottinghamshire, NG12 5GG, UK

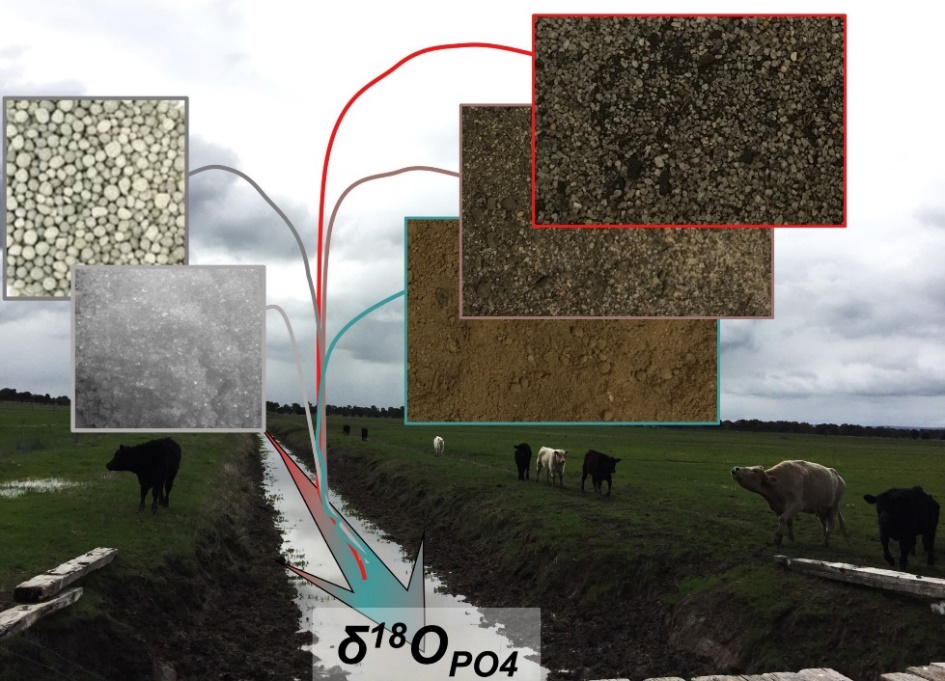
\*Author for correspondence: naomi.wells@lincoln.ac.nz

Submitted to *Journal of Environmental Management*

**Highlights**

* Isotope fingerprints of soil and fertiliser PO43- (δ18OP) vary within catchments
* Source mixing and biological turnover affect δ18OP signatures exported downstream
* Tracing agricultural pollution with δ18OP requires accounting for soil zone dynamics

**Abstract**

****Accurately tracing the sources and fate of excess PO43- in waterways is necessary for sustainable catchment management. The natural abundance isotopic composition of O in PO43- (δ18OP) is a promising tracer of point source pollution, but its ability to track diffuse agricultural pollution is unclear. We tested the hypothesis that δ18OP could distinguish between agricultural PO43- sources by measuring the integrated δ18OP composition and P speciation of contrasting inorganic fertilisers (compound v rock) and soil textures (sand, loam, clay). δ18OP composition differed between the three soil textures sampled across six working livestock farms: sandy soils had lower overall δ18OP values (21 ± 1 ‰) than the loams (23 ± 1 ‰), which corresponded with a smaller, but more readily leachable, PO43- pool. Fertilisers had greater δ18OP variability (~8‰) driven by both fertiliser type and manufacturing year. Upscaling these values showed that ‘agricultural soil leaching’ δ18OP signatures could span from 18 – 25 ‰, and are influenced by both fertiliser type and the time between application and leaching. These findings emphasise the potential of δ18OP to untangle soil-fertiliser P dynamics under controlled conditions, but that its use to trace catchment-scale agricultural PO43- losses is limited by uncertainties in soil biological P cycling and its associated isotopic fractionation.

**Keywords:** Phosphate leaching, stable isotope tracers, eutrophication, diffuse agricultural pollution, Peel-Harvey catchment, δ18O-PO43-

**1. Introduction**

Population growth and agricultural intensification has doubled phosphorus (P) inputs to global rivers (Beusen et al., 2016). While point source (e.g., wastewater treatment plants) P pollution can be effectively managed, diffuse P export from agriculture remains a pernicious water quality threat (Haygarth et al., 2005). This is in part due to the difficulty tracing P from its origin (e.g., fertiliser application) through the soil zone (where multiple biological and abiotic reactions can occur) to the receiving waters (Melland et al., 2018). New tools to identify excess P transported from soils to waterways via leaching and overland flow (henceforth ‘export’) are required to mitigate aquatic ecosystem degradation from eutrophication (e.g., hypoxia, fish kills).

Calls to use the isotopic composition of oxygen within PO43- (δ18OP) as a P tracer date back >10 years (Davies et al., 2014; Gruau et al., 2005; Young et al., 2009). This stems from evidence that PO43- sources (wastewater, tap water, fertilisers) can have distinct δ18OP signatures (Gooddy et al., 2018; Gooddy et al., 2015; Granger et al., 2017b). Additionally, knowledge that intracellular reactions with phosphatase enzymes impart a predictable temperature-dependent equilibrium signature as oxygen is exchanged between PO43- and the surrounding water (Chang and Blake, 2015; Gross and Angert, 2015; Jaisi et al., 2011), means δ18OP can also indicate ecosystems P cycling efficiency (Paytan et al., 2017; Pistocchi et al., 2017). Numerous studies propose using δ18OP source and transformation data to constrain catchment-scale P pollution dynamics (Gooddy et al., 2016; Granger et al., 2017b; Ishida et al., 2019; Tonderski et al., 2017). However, models are limited by uncertainty around the δ18OP ‘signatures’ generated by different catchment P sources.

Controls on agricultural soil δ18OP values are poorly understood. This is a critical knowledge gap as agricultural soils can dominate catchment P exports (Metson et al., 2017). Previous reviews show soil δ18OP ranges from 11 – 25 ‰ (Tian et al., 2020), and that agricultural soil δ18OP tends towards the higher end of the range predicted for biological equilibration with long-term soil water trends (Granger et al., 2017a; Ishida et al., 2019; Polain et al., 2018; Tamburini et al., 2010; Tian et al., 2020). Current models propose that systems reflect ‘source’ δ18OP values (e.g., fertilisers) when PO43- is in excess of biological demand, and shift towards equilibrium when PO43- is limiting due to enhanced P recycling (Bauke, 2020). Yet δ18OP variability (~5‰ across a single paddock (Granger et al., 2017a)) suggests additional factors are at play. And if P limitation were the main determinant of δ18OP reaching equilibrium, soil δ18OP should correlate with PO43- concentration, but this is not typically observed (Granger et al., 2017a; Tamburini et al., 2010; Tian et al., 2020). Fertilisers themselves cause further difficulty for defining the ‘agricultural’ δ18OP range: fertiliser δ18OP composition is variable (Gruau et al., 2005), but could account for up to 80 % of agricultural soil PO43- exports (McLaren et al., 2016; Nash et al., 2019).

The aim of this study was to parameterise the potential of δ18OP to trace agricultural PO43- export at the catchment scale. We hypothesised that fertiliser inputs and soil P fertility combine to create unique δ18OP signatures. We tested this by measuring the δ18OP composition of contrasting fertilisers and soils across an 1,800 km2 catchment, then using mixing models to define the possible range of exported δ18OP created by variable management (fertiliser application), biology (PO43- turnover), and hydrology (time before leaching, equilibrium δ18OP values).

**2. Materials & Methods**

2.1 Site description

Soil and fertiliser samples were collected from the 1,800 km2 Peel-Harvey catchment in southwestern Australia (Supporting Information (SI) S1 for maps). The catchment is flat (slope: 0.0015), with negligible elevation or aspect differences. Soils are P deficient, but their P retention capacity varies with the underpinning geology: the alluvial soils have a clay texture and quickly chemically immobilise fertiliser P inputs, while P is easily exported from the sand textured soils formed on ancient dunes (Bolland and Allen, 1998; McArthur and Bettenay, 1974). The region has a Mediterranean climate: hot, dry summers (27°C, 190 mm rain) v cool, wet winters (18°C, 1,000 mm rain). Fertilising to compensate for P immobilisation (clays) or leaching (sands) has contributed to the hyper-eutrophication of the Peel-Harvey Estuary (Valesini et al., 2019). Pasture soils still contain twice the optimal P range and leach 140 T P y-1 (Rivers et al., 2013).

2.2 Sample collection

Fertiliser isotopic variability (δ18OP(fert)) was constrained by analysing synthetic P fertilisers from CSBP (Perth, Western Australia). These covered dominant fertiliser types: monoammonium phosphate (MAP), superphosphate (SP), and a proprietary compound fertiliser with 16% N, 9% P, 14% S (AG). All three are water soluble (Nash et al., 2019). AG and SP were obtained for five manufacturing years (2013-2017) and MAP from one year (2017). These fertilisers are the products available to farmers in the catchment, but the exact mix applied to the sampled plots is unknown.

Soil samples (0 – 10 cm) were collected from 21 paddocks with contrasting soil textures (clay, sand, loam) on six ~2 km2 farms across the catchment (SI S1). Sampling was timed to winter (July 2017) when soil PO43- export occurs (Summers et al., 1999). Management effects, including fertiliser contamination of the measured soil δ18OP composition (δ18OP(soil)), were minimised by selecting farms with the same land use (beef grazing) and vegetation (ryegrass/clover pasture) participating in a multi-year P fertiliser minimisation trial. Triplicate samples (0-10 cm) spaced 10 m apart were collected over three transects from each paddock, and triplicates bulked to produce three samples per paddock, which were homogenised, sieved, and air dried. Around this period ten precipitation events were sampled for oxygen isotopes in water (δ18OH2O).

2.3 Sample analyses

All 63 soils (21 paddocks x 3 replicates) were analysed for pH, organic matter, and P concentration. A subset of 25 soils, selected to cover the different farms and textures and using P concentration to identify representative samples, were analysed for δ18OP(soil).

Soil pH was measured in 2.5:1 deionised water:dry soil extracts. Soil organic matter was determined via ignition (550 C for 4 h), and total (Ptotal) and organic (Porg) P concentration measured by extracting ignited v un-ignited soils with 1M H2SO4 (50:1) and measuring filtered extractant P concentration via ICP (Saunders and Williams, 1955). Because chemical bonding between PO43- and the soil matrix means that the amount of PO43- in Ptotal may not correspond to the amount of biologically available or leachable P, PO43- concentrations were additionally measured in sequential extractions as per (Hedley et al., 1982) in order to parameterise potential export and turnover rates. This defines PO43- by decreasing extractability as a proxy for availability (Gu and Margenot, 2020). Briefly, 2 g dry soil were extracted with 40 ml deionised water, 0.5M NaHCO3 (pH 8.5), 0.1M NaOH, and 1M HCl. Tubes were agitated for 18 h (rotary shaker), centrifuged (15 minutes), filtered (Whatman 0.45 µm) into duplicate 12 ml vials, stored at 4°C, and PO43- concentrations analysed via flow injection analysis after neutralising NaOH and NaHCO3 extracts.

δ18OP(soil) was measured on the total PO43- extractable with 1M HCl (PTIP). This enabled us to directly compare values across strongly contrasting soil textures, as preliminary tests showed clays had insufficient H2O extractable PO43- for δ18OP analyses, while sands had insufficient PO43- in the more tightly bound fractions. Using PTIP is also advantageous because, by capturing the majority of soil PO43-, it integrates the daily/seasonal P fluctuations observed in the more easily extracted fractions (Angert et al., 2011). So while sequential chemical extractions are useful indicators of the amount of soil PO43- likely to be exported (Rupp et al., 2018) the PTIP δ18OP(soil) provides a more robust and scalable soil ‘fingerprint’: extracted PO43- ‘fractions’ not actually exist in soils as discrete pools (Gu and Margenot, 2020) and do not reflect the potential biological recycling over export-relevant timeframes (Helfenstein et al., 2020; Wang et al., 2021).

The δ18OP compositions of soils (*n* = 25) and fertilisers (*n* = 11) were measured following Tamburini et al. (2010) Extractions were carried out at BGS (Wallingford) and isotope analyses at BGS (Keyworth). Briefly, 25 g dry soil (or 2 g fertiliser) were extracted overnight with 100 ml 1M HCl, centrifuged, and filtered. Dissolved organic matter was removed with DAX resin (20 ml), then ammonium phospho-molybdate precipitated with 4.2M ammonium nitrate and ammonium molybdate (dissolved in ammonium citrate) and re-precipitated as magnesium ammonium phosphate. After removing cations (AG50 X8 resin), silver phosphate (Ag3PO4) was precipitated using 5 ml of silver ammine solution. Triplicate subsamples (300 μg ) of the produced Ag3PO4 were weighed into silver capsules and the δ18OP composition determined via thermal conversion elemental analyser (TC-EA, ThermoFinnigan, Germany) at 1400°C with graphite and glassy carbon chips, coupled to a Delta+XL mass spectrometer (ThermoFinnigan, Germany). Triplicates’ precision was ≤0.3‰. Sample CO yield relative to Ag3PO4 standards was checked to ensure deviations <10%. δ18OP values were calculated with an internal Ag3PO4 standard, ALFA-1 (δ18O: 14.2‰). There are no international reference materials, so ALFA-1 was calibrated to the Ag3PO4 standard ‘B2207’ (Elemental Microanalysis Ltd.) from an inter-laboratory comparison. Oxygen isotope (18/16O) values are reported in δ ‰ v VSMOW.

2.4 Calculations

Soil organic carbon (Corg) was estimated as 0.516×loss-on-ignition (Jensen et al., 2018). Mineralisation of Porg to PO43-, which can affect δ18OP(soil) values (Gross and Angert, 2015), was parameterised as Pmin(14) (net mineralisation over 14 days) (Achat et al., 2010) based on measured soil organic v inorganic P composition (see SI S2). Data analyses were performed using R.v4.0 / RStudio.v1.3.959. Differences between farms and soil textures were determined via one-way ANOVA with an estimated marginal means post-hoc (Bonferroni adjusted), and correlations between soil parameters via Pearsons test (Kassambara, 2020). Figures were produced using ggplot2, patchwork, and munsell (Pedersen, 2019; Wickham, 2018; Wickham, 2016). Significance is defined as *p<*0.05 and values are reported as mean ± standard deviation.

2.4.1 Equilibrium δ18OP

δ18OP values produced due to equilibrium fractionation during extracellular P cycling (δ18OP(eq)) were calculated using Eq. 1, as per (Chang and Blake, 2015; Hacker et al., 2019):

(Eq. 1)

where δ18OP(eq) is defined by temperature (T, in kelvin) and δ18OH2O. Eq. 1 was solved two ways. First, because the PTIP used for δ18OP(soil) likely integrates long-term site conditions (Helfenstein et al., 2018), δ18OP(eq) was calculated using long-term records of soil temperature at 10 cm (mean: 21°C, high: 26°C, low: 18°C) and δ18OH2O (mean: -3.96 ‰, low: -2.74 ‰, high: -5.18 ‰, based on monthly precipitation δ18OH2O and amounts 1986-2012 for Perth, WA (Hollins et al., 2018; IAEA/WMO, 2020)). Second, because loosely bound PO43- in the sandy soils could be turning over daily🡪seasonal intervals, δ18OP(eq) was also calculated using modelled daily winter soil temperatures (mean: 13ºC, high: 21ºC, low: 8.2ºC) (Kearney, 2019) and precipitation δ18OH2O values measured during sampling (δ18OH2O: -3.07 ± 2 ‰, *n* = 10). Precipitation δ18OH2O was converted to soil water δ18OH2O based on evidence that soil δ18OH2O is a mass balance of seasonal precipitation (Benettin et al., 2018), ± +3‰ evaporative enrichment (Sprenger et al., 2017; Wan and Liu, 2016). See SI S3 for input data.

2.4.2 Export models

The possible δ18OP range exported from farms to waterways (δ18OP(export)) was determined using a two end-member mixing model that considered a range of fertilisers (type and application rate), times between fertiliser application and PO43- export, and soil biological P turnover (Fig. 1).

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**Fig. 1** Two-pool isotope mixing models (Eq. 2, Eq. 3) constrained the possible δ18OP range of PO43- exported (leaching, run-off) from fertilised soils (δ18OP(export)). The model was solved using recommended low, moderate, and high fertiliser applications rate (Pfert, in µg P g-1 soil) for each soil texture (clay, loam, sand) and δ18OP(fert) values for two fertilisers (AG: N-P-K, SP: superphosphate) manufactured between 2013 and 2017. δ18OP(fert) for each year × fertiliser were ‘mixed’ with each soil texture using the measured δ18OP(soil) range for PTIP and Psoil (µg P g-1 soil), defined by H2O extractable PO43- for fast export scenarios (a, c) and H2O + NaHCO3 extractable PO43- for slow/seasonal export scenarios (b, c). δ18OP(export) for both fast and slow export was calculated with (c, d) and without (a, b) soil biological P turnover (*X*P), which shifts δ18OP(export) towards d18OP(eq) (Eq. 1). Fast export *X*P (c) was approximated by [PH2O·e^(log(100+PH2O)/100·1)]/PTIP and slow export *X*P (d) by [PNaOH·e^(log(100+PNaOH)/100·1)]/PTIP. Arrows indicate the same values were applied across all soil textures, otherwise soil-specific values (mean±SD) were used. See SI S4 for model scripts.

The δ18OP(export) range was first defined assuming no biological turnover prior to export (Eq. 2):

(Eq. 2)

where *f*fert and *f*soil are the contribution of PO43- from fertiliser and soil, respectively, and δ18OP(fert) and δ18OP(soil) their isotopic composition; *f*fert was estimated for each soil texture based on its leachable soil P content (Psoil) and the recommended fertiliser application amount (Pfert). Two Psoil scenarios were considered: scenario a (fast), where export occurs within ~1 day of application (Psoil = H2O extractable PO43-), and scenario b (slow), where export occurs gradually over a whole season (Psoil = H2O + NaHCO3 extractable PO43- (Rupp et al., 2018)).

Next, scenarios a and b were rerun to consider biological P turnover pushing δ18OP values towards δ18OP(eq), as per (Helfenstein et al., 2018):

(Eq. 3)

where an exchange factor (*X*P) defines δ18OP(export.1) mixing with δ18OP(eq) (Gross and Angert, 2015). Eq. 3 constrains the effects of short-term (daily to monthly) biological P cycling, so δ18OP(eq) was defined based on diurnal variations in winter soil temperatures (Eq. 1). As *X*P is challenging to measure directly, values were approximated for each soil texture based on soil P mean residence time, calculated as the log-log linear relationship between H2O extractable PO43- and PO43- turnover in <1 hr, or, 2) NaOH extractable PO43- and PO43- turnover in >1 hr – 3 months (Helfenstein et al., 2020). For biologically active ‘fast’ export (scenario c), *X*P was defined as PO43- exchange in < 1 hr and applied to scenario a δ18OP(export.1) values. For biologically active ‘slow’ export (scenario d), *X*P was defined as the proportion of PO43- exchange in 1 hr – 3 months and applied to scenario b δ18OP(export.1) values.

δ18OP(fert) variability was parameterised two ways. First, models were run using the annual differences in δ18OP(fert) measured 2013-2017 for different fertiliser types (AG and SP, but not MAP because only one year was sampled). Second, Pfert was varied to reflect the low, high, and median fertiliser application rates recommended for each soil texture: 14, 58, 37 kg P ha-1 (clay), 18, 37, 28 kg P ha-1 (loam), and 9, 13, 11 kg P ha-1 (sand) (Summers and Weaver, 2011). Application rates (kg P ha-1) were converted to concentrations (µg P g-1) in the top 10 cm of soil (Pfert) using regional pasture soil bulk density (Viscarra Rossel et al., 2014): 1.44 ± 0.2 kg ha-1 (clay), 1.25 ± 0.2 kg ha-1 (loam), and 1.33 ± 0.1 kg ha-1 (sand). This model does not consider the complex soil chemical processes affecting long-term fertiliser mobility, meaning fertiliser contributions to ‘slow’ export scenarios (b, d) may be overestimated.

Variability in soil inputs was parameterised by solving each export scenario (a: fast, b: slow, c: fast + turnover, d: slow + turnover) using the mean, mean+SD, and mean-SD of δ18OP(soil) and Psoil for each soil texture, as well as for δ18OP(eq) (Henry and Wickham, 2020): *f*fert was calculated for each Psoil and Pfert combination, the minimum, mean-SD, mean, mean+SD, and maximum *f*fert for scenarios (a, b) × soil texture used to solve Eq. 2 for each fertiliser × manufacturing year, and then δ18OP(export.1) values used to solve Eq. 3 for scenario (c, d) × soil texture for each fertiliser × manufacturing year (Fig. 1). Output δ18OP(export) ranges were upscaled to possible ‘agricultural soil’ signatures based on the measured PO43- content and spatial coverage of soil textures for two sub-catchments with contrasting soil distributions, see SI Fig. S2 (McArthur and Bettenay, 1974; Weller, 2019). Upscaling calculations varied the contribution of AG v SP fertilisers and timing between fertilisation and export (a: fast v d: slow + turnover).

**3. Results**

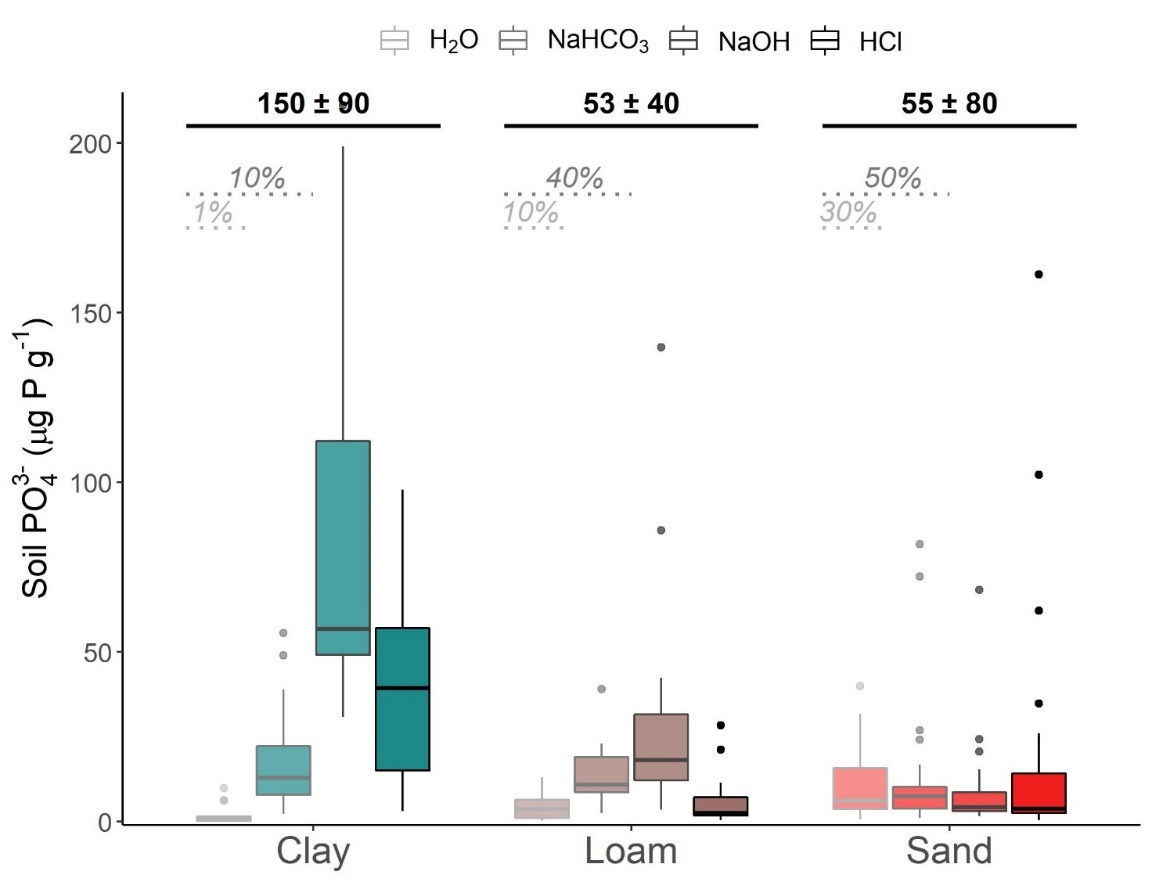
3.1 Fertilisers

Fertiliser types had different δ18OP(fert) values (*p<*0.05, F = 52). Values ranged from 17 ± 1 ‰ (SP) to 22 ± 0.05 ‰ (MAP) (Table 2). The δ18OP(fert) composition of SP and AG varied between manufacturing years: SP from 16 ± 0.2 ‰ in 2015 to 19 ± 0.2 ‰ in 2013, and AG from 20 ± 0.4 ‰ in 2014 to 22 ± 0.01 ‰ in 2015.

* 1. Soils

Soil pH was higher in clays than in loams or sands (*p<*0.05, F = 5.2) (Table 1). Corg was higher in clays (66 ± 20 mg C g-1) than loams (40 ± 10 mg C g-1) and sands (38 ± 30 mg C g-1) (F = 2.7, *p<*0.05) (Table 1), as was Ptotal (clay: 360 ± 20 µg g-1, loam: 190 ± 90 µg g-1, sand: 120 ± 100 µg g-1) (F = 20, *p*<0.05). Porg did not differ between soil textures or farms (SI Table S1), so Porg accounted for a higher proportion of Ptotal in sands (57 ± 10 %) than loams (45 ± 10 %) and clays (41 ± 0.09 %) (F = 12, *p<*0.01). The Corg:Porg ratio was higher in sands (630 ± 500 g/g) than in clays (540 ± 300 g/g) or loams (530 ± 200 g/g) (F = 3.3, *p<*0.01; Table 1). Pmin(14) was highest in absolute (F = 10, *p<*0.05) terms in sands (SI Table S3), and decreased as a proportion of PTIP from sands (14 ± 10 mg g-1) to loams (4.4 ± 3 mg g-1) to clays (0.74 ± 0.4 mg g-1) (F = 23, *p<*0.001; Table 1).

PTIP (based on H2O+NaHCO3+NaOH+HCl fractions) was higher in clays (150 ± 90 µg P g-1) than loams (53 ± 50 µg P g-1) or sands (55 ± 80 µg P g-1) (F = 11, *p<*0.05; Fig. 2). Water extractable PO43- differed between soil textures (F = 9.6, *p<*0.05), with concentrations in sands (11 ±10 µg P g-1) higher than in loams (4.2 ± 3 µg P g-1) and clays (1.7 ± 2 µg P g-1) (Fig. 2). NaHCO3 extractable PO43- did not differ between soil textures, but differed between farms (F = 3.0, *p =* 0.019): heavy clay soils in F6 had the lowest concentrations (7.6 ± 4 µg P g-1) and the predominantly sand soils in F1 had the highest (27 ± 20 µg P g-1) (see SI S2 for farm-level data). Clays had higher NaOH extractable PO43- (89 ± 50 µg P g-1) than loams (29 ± 30 µg P g-1) or sands (9.5 ± 10 µg P g-1) (F = 26, *p<*0.001). Likewise, HCl extractable PO43- was the highest in clays (39 ± 20 µg P g-1) and the lowest in loams (6.0 ± 8 µg P g-1) (F = 3.9, *p<*0.05). 30% of PTIP was H2O extractable in sands, versus 10% in loams and 1% in clays (F = 28, *p<*0.001; Fig. 2). The proportion of PTIP in the H2O+NaHCO3 fraction also decreased from sands (54 ± 20 %) to loams (41 ± 20 %) to clays (13 ± 5 %) (F = 40, *p<*0.001; Fig. 2). *X*P (Eq. 3) estimated for <1 hr was 30% (sand), 10% (loam), and 1% (clay), while *X*P estimated for turnover >1 hr – 3 months was 20% (sand), 60% (loam), and 90% (clay), see SI Table S7.



**Fig. 2** Phosphate in surface soils (0 – 10 cm) of 21 pastures with different soil textures in the Peel-Harvey catchment (Western Australia) based on sequential extraction with H2O (left, light outline), NaHCO3, NaOH, and HCl (right, dark outline). PTIP concentrations (sum of four fractions) for each soil textures is indicated at the top, and the percentage contribution of H2O (easily leachable) and H2O+NaHCO3 (seasonally leachable) fractions indicated with dashed lines. Boxes represent median ± 1 SD.

δ18OP(soil) values ranged from 25.3‰ (F4 loam) to 17.9‰ (F2 sand). Values negatively correlated with Pmin(14) (*p* = 0.03, *r* = -0.45) and positively correlated with Corg (*p* = 0.03, *r* = 0.5). Soil P concentrations did not correlate with δ18OP(soil). δ18OP(soil) values differed between soil textures but not farms, and were higher in loams (23.2 ± 1 ‰) than clays (22.3 ± 0.9 ‰) or sands (21.4 ± 2 ‰) (*p<*0.05; F = 3.8; Fig. 3). δ18OP(eq) values calculated using long-term temperature and δ18OH2O records ranged from 16.9 to 23.8 ‰ (20.4 ± 1.97 ‰), versus from 19.3 – 24.1 ‰ based on winter soil temperatures and δ18OH2O (Fig. 3). This places loam δ18OP(soil) values at or above the maximum δ18OP(eq) range, versus sand δ18OP(soil) values around mean δ18OP(eq).

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**Fig. 3** The δ18OP of PTIP in pasture soils (0-10 cm) classed as either clay, loam, or sand from six farms across the Peel-Harvey catchment (Western Australia). Boxes represent median ± 1 SD for each soil textures. Black lines represent the mean (solid line), ±1 SD (dashed lines), and minimum/maximum (dotted lines) of the long-term local δ18OP(eq) range (Eq. 2); the grey line indicates the mean δ18OP(eq) calculated for conditions during the winter sampling (*eq-w*).

3.3 Export model

For scenario (a), *f*fert (Eq. 2) decreased from 0.93 ± 0.1 (clays) via 0.84 ± 0.1 (loams) to 0.54 ± 0.2 (sands) (Fig. 4a). For scenario (b), *f*fert was 0.57 ± 0.1 for clays, 0.57 ± 0.1 for loams, and 0.37 ± 0.2 for sands (Fig. 4b). In scenario (a) δ18OP(export) values track δ18OP(fert), with clear differences between AG v SP applied to all soil textures (Fig. 4c). Rapid P turnover (scenario c) shifted sand, but not clays or loams, δ18OP(export) away from low-end δ18OP(fert) values (Fig. 4e). Yearly δ18OP(fert) differences in SP (2013 v 2014-2017) and AG (2014 v 2015) fertilisers affected modelled δ18OP(export) from clays and loams, but not sands, under ‘fast’ scenarios (a, c) (Fig. 4c,e). For ‘slow’ scenarios (b, d), differences in SP v AG δ18OP(export) values were only expressed when *X*P = 0% (Fig. 4d), and δ18OP(export) from all soil textures and fertilisers normalised to δ18OP(eq) with ~monthly P turnover (Fig. 4f). Upscaling to sub-catchments, the possible δ18OP(export) range is narrowest (~2‰) if export is slow and fertilisation type uniform (Table 3). In both sub-catchments fertiliser mixing was more important than the export speed in defining the δ18OP(export) values, and mixed fertilisers + slow export produced the widest possible δ18OP(export) range.

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**Fig. 4** The possible range of the isotopic composition of PO43- export from clay, loam, and sand pasture soils (δ18OP(export), ‰ v. VSMOW) within a catchment depends on fertiliser contribution to the leachable soil PO43- pool (*f*fert) and fertiliser δ18OP composition (AG: black circles, SP: grey triangles, manufactured 2013-2017). δ18OP(export) values were calculated for two export scenarios: fast (a, c, e), where PO43- is exported <1 day after fertilisation, and slow (b, d, f), where PO43- is leached over weeks-months. Both fast and slow export could occur with (e, f: *X*P = 1 h or 1 month) or without (c, d: *X*P = nil) soil biological P turnover (Eq. 3). Violins (a, b) show the distribution of *f*fert values around the mean (solid line); boxes (c-f) show the mean ± 1 SD for δ18OP(export), with whiskers to the minimum and maximum. Box colours distinguish soil textures (as defined in a and b) and outlines the fertiliser (AG: black, SP: grey).

**4. Discussion**

4.1 Fertilisers

The δ18OP(fert) range here (17 – 21 ‰) fits previous reports for inorganic commercial fertilisers (Table 2). Variations in δ18OP(fert) are generally attributed to geologic differences in the rocks sourced to make the fertilisers (Davies et al., 2014; Gruau et al., 2005). This is because the δ18OP composition of the sedimentary rocks sourced to produce PO43- fertilisers depend on age and/or equilibration with δ18OH2O during formation (Sun et al., 2020). Here the ~8 ‰ difference between N-bearing (AG, MAP) and rock (SP) fertilisers corresponded with different geologic source materials: AG and MAP were manufactured using materials from Florida, USA (Eocene – Miocene, ~55 MBP (Trueman, 1965)), while SP was manufactured using Christmas Island rocks (Oligocene – Pliocene, ~33 MBP (Van Kauwenbergh et al., 1990)). However, this explanation for the 5 - 8 ‰ difference between the fertiliser types does not hold up to scrutiny. The ~20 MY gap is negligible in geologic time (e.g., the 3 ‰ difference between PO43- in China v the Middle East corresponds to ~300 MY (Sun et al., 2020)). Likewise, different δ18OP(eq) during formation is unlikely given similarities in the δ18OH2O and temperature regimens between the Indian Ocean and tropical Atlantic (LeGrande and Schmidt, 2006). This suggests that there is an additional factor than the commonly cited ‘geologic δ18OP differences’ that is contributing to the consistent offset between fertiliser types. We note that geology-driven variations in δ18OP(fert) is not robustly supported by the literature, with source material origins provided in only four of nine published studies (Table 2). This suggests that future work should encompass isotopic fractionation during manufacturing, which is theoretically possible given the filtration and solubilisation processes used (Chien et al., 2011), especially given the consistent differences between fertilisers made from raw (SP) v pre-processed (AG, MAP) materials. A similar mechanism was proposed to explain differences in tap water δ18OP (Gooddy et al., 2015), and requires further consideration.

Regardless of the exact driver (source, manufacturing), a single precise δ18OP(fert) value is unlikely to exist at the spatial and temporal scale of catchment studies. Establishing methods for predicting, and thus better constraining, δ18OP(fert) will be critical to any future attempts to use δ18OP to trace aquatic PO43-. As first steps, we recommend future isotope studies report both the chemical form and geologic (rather than commercial) origin of P fertilisers.

4.2 Soils

Soil P variations fit expectations (Table 1, Fig. 2). PTIP content was at the very low end for agricultural soils and Corg:Porg ratios at the high end for mineral soils, both typical for weathered southwestern Australian soils (Helfenstein et al., 2020; Spohn, 2020; Turner and Laliberte, 2015). Phosphate partitioning followed the anticipated shift from sands with low, highly leachable, PO43- pools, to clays with larger, less leachable, PO43- pools (Nash et al., 2019; O'Halloran et al., 1987). These soil texture differences provide a solid basis to test how P buffering capacity controls δ18OP(soil) and δ18OP(export).

δ18OP(soil) is hypothesised to reflect differences in the size and availability of soil PO43- (Bauke, 2020). Loosely bound PO43- (H2O or NaHCO3 fractions) can be completely recycled in days, whereas more tightly bound PO43- (NaOH or HCl fractions) turnover may take centuries (Helfenstein et al., 2020). Because biological PO43- turnover moves δ18OP(soil) towards δ18OP(eq), more labile PO43- fractions tends to have (higher) δ18OP values closer to δ18OP(eq) and more tightly bound PO43- fractions tend to have (lower) δ18OP(soil) values closer to the geologic parent material δ18OP (Roberts et al., 2015; Tian et al., 2020). This predicts that the sands’ predominantly labile PO43- pool would shift δ18OP(soil) values higher than the clays, where most PO43- is tightly bound (Rodionov et al., 2020). Instead, the sands had the lowest δ18OP(soil) values, and almost all δ18OP(soil) values fell within the δ18OP(eq) range (Fig. 3). It is reasonable that all soil PO43- was within the δ18OP(eq) range as geologic PO43- is unlikely to persist in any of these ~300,000 year old soils (Shen et al., 2020; Turner and Laliberte, 2015). But if PO43- is in isotopic equilibrium with soil water, why do δ18OP(soil) values fall into distinct ‘soil texture’ zones within this range?

Variations within the δ18OP(eq) range could be driven by three factors: divergent equilibrium conditions (soil temperature, δ18OH2O), different PO43- sources, and/or fractionation by competing biological processes. First, daily – seasonal parameter fluctuations are not seen to affect δ18OP(soil) of PTIP (Angert et al., 2011; Lei et al., 2019). This suggests that long-term evaporation (δ18OH2O) or temperature differences between the soil textures would be needed to create a ‘soil specific’ δ18OP(eq) range. Factors like slope, aspect, and vegetation (Hacker et al., 2019; Sprenger et al., 2016) are excluded here due to the flat terrain and relatively homogenous land-use, but soil texture can affect evaporation. However, a textural impact on evaporation would elevate δ18OP(soil) in coarse grained sands above δ18OP(soil) in the fine grained clays (Gazis and Feng, 2004), the opposite of the observed pattern. Second, pastures receive Porg and PO43- inputs. Inorganic fertilisers can be ruled out as δ18OP(fert) values were lower than loam δ18OP(soil) (Fig. 4), and likewise processed Porg (manure) likely has δ18OP below the δ18OP(soil) range here (Granger et al., 2017b). While raw Porg inputs (plants) can have δ18OP up to ~30 ‰ (Pfahler et al., 2013; von Sperber et al., 2015), the mechanism through which they could differently affect soil textures under similar management (including pasture plants) is unclear. Charred organic matter is also a potentially significant P input (Baldock et al., 2013). A survey of nearby pastures suggests that loams contain more char than sands or clays (9.2 ± 2 mg C g-1 v 7.7 ± 2 mg C g-1 and 4.9 ± 2 mg C g-1, respectively) (Viscarra Rossel et al., 2014). If char contains 20 µg PO43--P g-1 (Pluchon et al., 2015), this could be the source of 30% of loam PO43-, v 5% of clay PO43-. This is an intriguing possibility, but measurements of combusted organic material suggest char δ18OP values may be too low (~15 ‰) (Bigio and Angert, 2019) to explain the observed loam δ18OP(soil) values.

Alternatively, both the mineralisation of Porg to PO43- and microbial PO43- assimilation affect δ18OP. Scavenging Porg in low fertility soils can decrease δ18OP(soil) below δ18OP(eq) (Liang and Blake, 2006; Pistocchi et al., 2020), and estimates suggest that mineralisation is highest (Pmin = ~1% of PTIP per fortnight) in the P-poor, relatively low δ18OP(soil) sands (Table 1). Additionally, microbial PO43- assimilation increases δ18OP(soil) (Blake et al., 2005), with stronger fractionation when P is limiting (Lis et al., 2019). This gives a plausible explanation for the relatively high loam δ18OP(soil) values. Low P in both sands and loams could promote microbial PO43- uptake and increase δ18OP(soil) of both soil textures (Bünemann et al., 2012), but the low sand Corg and P content causes its microbial P to be more efficiently recycled and δ18OP(soil) reset to the mean δ18OP(eq) range. This supports the assumption in catchment models that sand PO43- is completely exhausted every winter (Summers et al., 1999). Although the exact driver of the soil textures δ18OP(soil) patterns is not certain, the non-random distribution of δ18OP(soil) within the δ18OP(eq) range emphasises the need to move beyond simply defining δ18OP(soil) as ‘in’ or ‘out’ of equilibrium and start unpicking the competing biological and hydrologic processes at play.

4.3 δ18OP as a tracer of PO43- export from agricultural systems

There are three main questions about agricultural P export that δ18OP models look to answer: 1) how much fertiliser is exported directly to water?, 2) which landscape units contribute disproportionately to PO43- export?, and, 3) how much does agriculture contribute to catchment PO43- loads? The mixing models used here generated clear constraints on how δ18OP data could be used at each of these scales.

Directly exported P fertilisers are a significant financial and environmental risk. Although difficult to measure, estimates suggest fertilisers account for 30-80% of PO43- leached from agricultural systems (Nash et al., 2019), and radiotracer studies show 20-30 % of fertiliser PO43- is leached from pastures within two months of application (McLaren et al., 2017; McLaren et al., 2016). There is further uncertainty about the timing of these direct export events: how long fertilisers stay in granular form depends on rainfall, temperature, and fertiliser type (McLaren et al., 2017). The wide δ18OP(fert) range reported here indicates that δ18OP values could prove a uniquely powerful tool for untangling these PO43- leaching dynamics at the plot - paddock scale if isotopically distinct fertiliser v soils were first identified. Yet the same δ18OP(fert) range complicates efforts to identify soil and land-use specific δ18OP(export) signatures (Fig. 4, Table 3).

The twin possibilities of mixed fertiliser use and variable biological P turnover drive the uncertainty in δ18OP(export). Across the modelled two sub-catchments the ‘agricultural’ δ18OP(export) could reasonably range between 18‰ and 25‰, a much wider range than would be predicted by simply using the sub-catchment soil maps to upscale δ18OP(soil). This level of uncertainty means large datasets of receiving water δ18OP values are needed to generate statistically robust identification of the fertiliser and soil PO43- sources. For instance, measuring an ‘out of equilibrium’ downstream δ18OP value of 19‰ could reasonably be evidence of SP export, but would not rule out export of other fertilisers contributing up to 40% of PO43-. Conversely, measuring a δ18OP value of 21‰, well above the SP δ18OP(fert) range, could not conclusively rule SP out as a PO43- source (Fig. 4, Table 3). So while biological P turnover could ameliorate some of the variability created by fertiliser-soil mixing by shifting δ18OP(export) values towards δ18OP(eq), it also highlights more intransigent sources of uncertainty. First, the soil δ18OP(eq) range is itself uncertain due to questions around the extent to which variations are caused by hydrology (temperature and δ18OH2O (Benettin et al., 2018; Skrzypek et al., 2019)) v biology (balance between biological P cycling pathways (Helfenstein et al., 2018; Siegenthaler et al., 2020)). Second, evaluating these questions about soil δ18OP(eq) dynamics is complicated by the fact that the PO43- pools that can be extracted for δ18OP analysis do not necessarily align with those that are environmentally relevant (Gu and Margenot, 2020; McConnell et al., 2020). Both situations contrast with the established approaches to tracing PO43- pollution point sources like wastewater effluent (Gooddy et al., 2018), where biological modification of the defined source signature will occur post export to the waterway (Davies et al., 2014). The interconnected uncertainties about P turnover and δ18OP(eq) must be resolved in order to usefully incorporate δ18OP into P reactive transport models (Dorioz et al., 1998). One potential is that improved δ18OP(eq) understanding could be used to construct δ18OP catchment models based on temperature and δ18OH2O regimens.

**5. Conclusions**

The ability of phosphate isotopes (δ18OP) to trace diffuse agricultural pollutants through catchments is limited by variations in soil zone inputs and reactions. The analytical template here highlights the importance, but also the limitations, of using site-specific δ18OP(fert) values to identify diffuse agricultural pollution. Uncertainty from δ18OP can reasonably be constrained via site-specific measurements in smaller catchments, but until biological turnover (fractionation and rates) is better defined surface water δ18OP signatures should be attributed to diffuse catchment sources with caution.

**Associated content:** The Supporting Information pdf contains additional: S1) site maps (Figure S1: Site map with farm locations, Figure S2: Sub-catchment maps with soil textures), S2) additional soil data (Table S1: Background data on soil P status and N content, Table S2: Sequential extraction soil PO43- concentration information by farm × soil texture, Table S3: Pmin(14) data), S3) input variables for δ18OP(eq) and mixing model calculations (Table S4: precipitation δ18OH2O for winter 2017, Table S5: Long-term soil temperature data, Table S6: Modelled daily winter soil temperatures, Table S7: Estimated P turnover (*X*P) by soil texture), and, S4) R scripts (S4.1: δ18OP(eq) calculations,S4.2: mixing models, S4.3: up-scaling calculations). Soil data are available on <https://figshare.com/s/e1416e6217fe0e7f3b10> (*link for review only, will be published with DOI upon acceptance*).

**Acknowledgements:** Iain Alexander and Natasha Carlson-Perret (Southern Cross University) assisted with soil P extractions. Robert Summers (Department of Primary Industries and Regional Development, Western Australia) supplied background site data, soil sampling equipment, and fertiliser samples. Fiona Valesini (Murdoch University) helped organise field work and secure research funding. Thanks to Justin Mercy (CSBP) for information on fertiliser sources and production, and to the six land owners for granting access to their properties. Research was funded by Australian Research Council grant LP150100451 and by the UK’s Natural Environment Research Council Environmental Isotope Facility grant IP-1664-1116.

**Author contributions:** NSW, MYR, and BDE designed the study. NSW and MYR carried out the study. PJW and ACS carried out isotopic extractions and analysed the samples. NSW and DCG analysed the data. NSW and DCG wrote the manuscript, with input from all co-authors.

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**Tables**

**Table 1:** Characteristics of pasture soils (0 – 10 cm) with contrasting textures (sand, clay, loam) collected from six farms (F1 – F6) across the coastal Peel-Harvey catchment in southwestern Western Australia (see SI S1 maps). Sample numbers indicate the total bulked (*n* = 3) cores collected for P content and the subset analysed for δ18OP(soil). The contribution of Porg to Ptotal is calculated on a g/g basis. Potential P mineralisation over 14 days (Pmin(14)) is reported relative to the total HCl extractable PO43- concentration (PTIP). See SI S2 for additional soil chemistry data.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Texture** | **Farm** | **sample #s** | | **pH** | **Corg**  *mg C g-1* | **Ptotal**  *µg P g-1* | **% Porg**  *(Porg/Ptotal)* ·*100* | **Corg:Porg**  g/g | Pmin(14):PTIP  mg/g |
| *all* | *δ18OP* |
| **Clay** | F3 | 3 | 1 | 5.8 (0.1) | 33 | 390 (70) | 33 (9) | 290 (100) | 0.27 (0.07) |
| F4 | 9 | 4 | 6.5 (0.3) | 68 (20) | 450 (200) | 37 (7) | 440 (100) | 0.68 (0.3) |
| F6 | 9 | 3 | 6.3 (0.1) | 76 (20) | 250 (60) | 46 (7) | 700 (300) | 0.90 (0.4) |
| **Loam** | F2 | 6 | 4 | 6.1 (0.09) | 34 (9) | 150 (50) | 41 (7) | 590 (200) | 6.6 (4) |
| F4 | 3 | 2 | 6.4 (0.06) | 58 (20) | 320 (100) | 37 (10) | 540 (200) | 1.9 (2) |
| F5 | 9 | 3 | 6.3 (0.2) | 38 (10) | 170 (50) | 51 (8) | 490 (200) | 3.9 (2) |
| **Sand** | F1 | 9 | 4 | 5.8 (0.3) | 46 (40) | 470 (600) | 53 (10) | 340 (200) | 7.7 (7) |
| F2 | 6 | 2 | 6.3 (0.09) | 54 (30) | 120 (80) | 55 (10) | 1000 (600) | 18 (10) |
| F3 | 9 | 2 | 6.2 (0.1) | 16 (7) | 63 (20) | 61 (20) | 510 (200) | 16 (10) |

**Table 2** Inorganic fertiliser δ18OP values reported for this study and others (δ18OP(fert), values in ‰ v VSMOW), with respect to fertiliser type, where the fertiliser was manufactured, and where the PO43- raw material was sourced from (‘unspecified’ denotes data unavailable).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Fertiliser type** | **Manufactured** | **Sourced** | **δ18OP(fert)** | **Reference** |
| Superphosphate | Australia | Christmas Island | 16.7 ± 1  *15.6 – 18.7* | This study |
| Europe | *Unspecified* | 17.7 ± 0.2 | Tamburini et al. (2010) |
| Japan | Japan | 12.7 | Ishida et al. (2019) |
| Australia | *Unspecified* | 21.4 ± 0.5 | Polain et al. (2018) |
| Israel | *Unspecified* | 21.8 ± 0.3 | (Gross and Angert, 2015) |
| Europe | Morocco & USA | 23 ± 0.3 | Gruau et al. (2005) |
| Monoammonium phosphate | Australia | USA | 21.6 ± 0.05 | This study |
| Australia | *Unspecified* | 20.2 ± 0.1 | Polain et al. (2018) |
| N-P-S-K | Australia | USA | 21.3 ± 1  *19.7 – 22.4* | This study |
| Europe | Morocco & USA | 21.8 ± 0.5 | Gruau et al. (2005) |
| Europe | *Unspecified* | 20.9 ± 6 | Granger et al. (2017b) |
| *Unspecified* | USA | USA | 23.8 ± 1 | Li et al. (2011) |
| USA | *Unspecified* | 19 ± 1 | McLaughlin et al. (2006) |
| USA | Israel | 19.6 | Young et al. (2009) |
| China | China | 11.5 ± 0.1 | Tian et al. (2020) |

**Table 3** Possible δ18OP(export) range from two sub-catchments with differing soil distributions (maps: SI S1). The δ18OP(export) range was calculated by varying the relative proportion of SP v AG fertilisers and speed of PO43- transport from soil to water (fast, scenario a: mixing with H2O extractable PO43- pool, *X*P = 0%; or scenario d: mixing with H2O+NaHCO3 extractable PO43-, *X*P = 20 – 90%, depending on soil texture), see SI S4 for calculations.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sub-catchment** | **δ18OP(export) range** | | |
| *Fertiliser* | *Transport* | |
| Fast+Slow1 🡪 Mostly slow2 | |
| Pinjarra  *19% clay, 4.4% loam, 76% sand* | 1 SP + 0 AG | 17.7 – 21.3 | 18.5 – 20.5 |
| 0.6 SP + 0.4 AG | 18.7 – 21.9 | 19.1 – 22.6 |
| Harvey  *21% clay, 29% loam, 50% sand* | 1 SP + 0 AG | 17.7 – 21.1 | 18.7 – 20.5 |
| 0.6 SP + 0.4 AG | 18.8 – 23.0 | 19.2 – 24.9 |

1 50% ‘d’ + 50% ‘a’

2 90% ‘d’ + 10% ‘a’

**Figure captions**

**Fig. 1** Two-pool isotope mixing models (Eq. 2, Eq. 3) constrained the possible δ18OP range of PO43- exported (leaching, run-off) from fertilised soils (δ18OP(export)). The model was solved using recommended low, moderate, and high fertiliser applications rate (Pfert, in µg P g-1 soil) for each soil texture (clay, loam, sand) and δ18OP(fert) values for two fertilisers (AG: N-P-K, SP: superphosphate) manufactured between 2013 and 2017. δ18OP(fert) for each year × fertiliser were ‘mixed’ with each soil texture using the measured δ18OP(soil) range for PTIP and Psoil (µg P g-1 soil), defined by H2O extractable PO43- for fast export scenarios (a, c) and H2O + NaHCO3 extractable PO43- for slow/seasonal export scenarios (b, c). δ18OP(export) for both fast and slow export was calculated with (c, d) and without (a, b) soil biological P turnover (*X*P), which shifts δ18OP(export) towards d18OP(eq) (Eq. 1). Fast export *X*P (c) was approximated by [PH2O·e^(log(100+PH2O)/100·1)]/PTIP and slow export *X*P (d) by [PNaOH·e^(log(100+PNaOH)/100·1)]/PTIP. Arrows indicate the same values were applied across all soil textures, otherwise soil-specific values (mean±SD) were used. See SI S4 for model scripts.

**Fig. 2** Phosphate in surface soils (0 – 10 cm) of 21 pastures with different textures in the Peel-Harvey catchment (Western Australia) based on sequential extraction with H2O (left, light outline), NaHCO3, NaOH, and HCl (right, dark outline). PTIP concentrations (sum of four fractions) for each soil texture is indicated at the top, and the percentage contribution of H2O (easily leachable) and H2O+NaHCO3 (seasonally leachable) fractions indicated with dashed lines. Boxes represent median ± 1 SD.

**Fig. 3** The δ18OP of PTIP in pasture soils (0-10 cm) classed as either clay, loam, or sand from six farms across the Peel-Harvey catchment (Western Australia). Boxes represent median ± 1 SD for each soil texture. Black lines represent the mean (solid line), ±1 SD (dashed lines), and minimum/maximum (dotted lines) of the long-term local δ18OP(eq) range (Eq. 2); the grey line indicates the mean δ18OP(eq) calculated for conditions during the winter sampling (*eq-w*).

**Fig. 4** The possible range of the isotopic composition of PO43- export from clay, loam, and sand pasture soils (δ18OP(export), ‰ v. VSMOW) within a catchment depends on fertiliser contribution to the leachable soil PO43- pool (*f*fert) and fertiliser δ18OP composition (AG: black circles, SP: grey triangles, manufactured 2013-2017). δ18OP(export) values were calculated for two export scenarios: fast (a, c, e), where PO43- is exported <1 day after fertilisation, and slow (b, d, f), where PO43- is leached over weeks-months. Both fast and slow export could occur with (e, f: *X*P = 1 h or 1 month) or without (c, d: *X*P = nil) soil biological P turnover (Eq. 3). Violins (a, b) show the distribution of *f*fert values around the mean (solid line); boxes (c-f) show the mean ± 1 SD for δ18OP(export), with whiskers to the minimum and maximum. Box colours distinguish soil textures (as defined in a and b) and outlines the fertiliser (AG: black, SP: grey).