

Biogeosciences
Manuscript bg-2017-307

Dear Editor Sönke Zaehle,

Thank you for your work on our manuscript. We are humbled with all the suggestions made by the reviewer. We would like to thank him/her again for this immense effort on reviewing our manuscript. Those insights and precisions strengthened in the most positive way our manuscript.

The changes made on the manuscript are briefly described in the following. We corrected all English problems noted by the reviewer. We cited the original papers instead of some more recent reviews. We added additional information, and adjusted the non-accurate statements following his/her directions. The citations regarding fractionation methods (e.g. Hedley) were reviewed, and exclusions were made when they were not appropriate (when referring to soil P composition). We changed nomenclature for “fractionation” or “fractions” when those citations were required on the text. We corrected the missing abbreviations for phosphorus, inorganic phosphorus, and organic phosphorus (P, P_i and P_o), except at the beginning of sentences, and when we described the search strategy on methods section. New references were added, and some were excluded following the reviewer. All the references were reviewed for formatting, and several details adjusted.

Responses to the reviewers' comments are described below and are indicated by “Response”. Changes made on the manuscript were all based on these responses. To facilitate the evaluation, we left the reviewers comments before each response.

Responses made by the authors are in bold and indicated with the term “Response”. After the responses to the reviewers comments one can find the manuscript with all changes marked.

Responses to the comments made by the Anonymous Referee #1

1. Writing quality: for the most part, the quality of English has improved. However, there are still some problems in some places, which are noted below.

Response: We followed and corrected all instances pointed out by the reviewer.

2. I am puzzled about some of the literature cited in the introduction and discussion, and still think there is a bias to citations of specific authors rather than a good overview of the literature.

a) Please cite original papers where possible, rather than recent review papers. For example, why cite Nash et al. (2014), rather than Newman and Tate 1980, *Commun. Soil Sci. Plant Anal* 11:835-842) or Tate and Newman (1982, *Soil Biol. Biochem* 14:191-196) for the description of P forms on p. 2, line 17? Why cite Turner 2008a for phosphatase and orthophosphate uptake (p. 2, line 20) – this was well-known prior to that paper. Why cite Huang et al. 2017 for vegetation and organisms (p. 10, l. 24) or mycorrhizae (p. 13, l. 16), when it presents no new information on either of these topics? The same is also true for Yu et al. 2013. There has been a lot of good research into many aspects of P cycling by a lot of good scientists; citing the review papers instead of the original research is unfair to the original researchers, and runs the risk of introducing errors into the literature if the original work was cited incorrectly in the review. Would the authors of this manuscript not prefer to have it cited directly?

Response: We completely agree with the reviewer. The two Newman and Tate's original papers were cited instead of Nash et al. (2014). Jackman and Black (1952) was used instead of Turner (2008a), which is one of the first studies to use enzyme additions to evaluate the availability of inositol hexaphosphate. Huang et al. 2017 was excluded from the second occasion, but it was maintained in the first one because we were not citing it for vegetation and organisms, but for the same struggle he had to draw conclusions from the influence of vegetation and organisms on the soil P composition (as determined by ³¹P NMR). Yu et al (2013) was excluded from the manuscript.

b) Please be careful citing studies using fractionation methods (e.g. Hedley) in study discussing specific chemical forms. Fractions from fractionation methods are operationally defined, not chemically defined, especially the Hedley fractionation method.

Response: The citations regarding fractionation methods were reviewed. Exclusions were made when they were not appropriate

(when referring to soil P composition), and we changed nomenclature for “fractionation” or “fractions” when they were required on the text.

3. Abstract:

Lines 11-12: “nuclear magnetic resonance of NaOH-EDTA extracts” should be “nuclear magnetic resonance spectroscopy of NaOH-EDTA extracts” (something I missed in my previous review).

Response: Alteration made.

Lines 16-17: “organic and inorganic P compounds variations” should be “variations in organic and inorganic P compounds”

Response: Alteration made.

Lines 19-20: “and after altogether with plant and microbe” I still do not understand what the authors are trying to say here; something seems to be missing. Do they mean “and together with plant and microbe”?

Response: or clarification, “and after altogether with” was cut off and we maintained “plant and microbe” only.

4. Introduction:

p. 2, line 6 and elsewhere: “phosphorus” should be “P” after the first use of the abbreviation, except at the start of a sentence.

Response: All occurrences of “phosphorus” were changed to “P”, except at the beginning of sentences, and when we described the search strategy on methods section.

p. 2, line 14: “composed by specific” should be “composed of specific”

Response: Alteration made.

p 2, lines 17 and 18: please replace “Nash et al. 2014” and “Cade-Menun and Preston 1996” with more appropriate references, because these compound classes were recognized prior to the publication of these papers

Response: The two Newman and Tate’s original papers were cited instead of Nash et al. (2014).

p. 2, line 20: “As most enzymes, the activity of soil P cycling enzymes” should be “As with most enzymes, the activities of soil phosphatases”

Response: Alteration made.

p. 2, line 21: “specific enzyme optimum” should be “specific enzyme optima”

Response: Alteration made.

p. 2, lines 24-25: DNA adsorption is only below 5 (the isoelectric point of DNA); also, why is a review paper (Yu et al. 2013) cited here, rather than one of the original references about pH? Please see the comments for page 12, below.

Response: “in pH lower than 5 (the isoelectric point of DNA)” was added to the sentence. Cai et al. 2006 was used instead of Yu et al. 2013.

p. 2, lines 27-31: studies using P fractionation (e.g. Walker and Syers 1976, Yang and Post 2011) do not give any information about Po and Pi forms and compounds, because fractionation by definition can only give information about operationally-defined pools. Please rewrite this paragraph, and cite better references that actually describe changes in Pi and Po compounds

Response: The new references were added to support the “forms and compounds” terms. The sentence was written again adding “for P fractions” when referring to fractionation methods.

p. 3, line 6: “While this study” should be “While the Feng et al. (2016) study”

Response: Alteration made.

p. 3, line 11: “Variation in” should be “Variations in”

Response: Alteration made.

p. 3, lines 27-28: “the NaOH-EDTA extraction does not separate Fe-, Al- and Ca-phosphate compounds (Kizewski et al. 2011)” although this might be what Kizewski et al. said, it isn’t very accurate. A better way to say it is: “the high pH of the NaOH-EDTA extraction separates P species from the cations (e.g. Al, Fe, Ca) with which they were associated in soil”.

Response: Text was changed to the suggested one.

p. 3, lines 28-30: Yes, there are other methods to study P dynamics and soil P composition, but none of these methods is perfect individually. For example, while XANES is a solid-state technique that does not require extraction, P concentrations are often below the detection limit, so it can only detect broad P species groups (e.g. Fe-P, Ca-P), but can’t for example say if DNA is sorbed to Fe or Al. The most thorough studies of soil P use a combination of techniques together, and not any single technique (e.g. Liu et al. 2013 J.

Environ. Qual 42:1763-1770; Liu et al. 2015 Environ Sci Technol 49:168-176).

Response: Our previous sentence was modified to include this explanation and references.

p. 3, lines 30-32: "This does not mean that the results on pyrophosphate, polyphosphate and total orthophosphate concentrations are not useful, however, there are other inorganic P compounds of importance in soils". This sentence makes no sense to me. No, there are no other inorganic P compound in soil other than orthophosphate, pyrophosphate and polyphosphate. However, as noted above, extraction with NaOH-EDTA removes inorganic (and organic) P compound from the cations with which they are associated in soils. Thus, as noted below, a combination of techniques will give the most complete picture of soil P speciation and dynamics. Please rewrite this sentence.

Response: As we added the previous explanation we excluded this unprecise sentence.

5. Methods: These are now clear and well-written.

6. Results:

p. 7, line 1: "there was no pH effect on both pools" should be "there was no pH effect on either pool"

Response: Alteration made.

p. 7, line 6: "additional Appendix S3" delete "additional"

Response: Alteration made. All "additional" were deleted from the manuscript when referring to appendices.

p. 7, line 8: "additional Appendix S6" delete "additional"

Response: Alteration made.

p. 9, lines 5-6: "organic and inorganic P" should be "Po and Pi" to be consistent with the rest of the manuscript

Response: All occasions were changed to the abbreviation, except at the beginning of sentences.

7. Discussion: In general, this part of the manuscript is improved compared to previous versions.

p. 10, line 5: “P organic and inorganic compounds” should be “Po and Pi compounds”

Response: Alteration made.

p. 10, line 8: “the decaying degree of C element is lower than the P” I do not understand what you are trying to say here. Is it “organic matter degrades more slowly than Po compounds”? Please rewrite.

Response: decaying degree means decomposition and this term was added in parenthesis after the mentioned terms for clarity.

p. 10, line 9: “Turner and Condrón 2013” is an opening paper introducing a special issue, and does not contain data to support this statement of fact. Please cite another reference that actually contains data.

Response: Turner and Condrón 2013 was excluded, Walker 1965 was maintained.

p. 10, lines 16-17: It is not possible to determine specific P compounds such as apatite with Hedley fractionation, and the long extraction times likely also degrade organic P. Please cite a better reference, with actual soil chemical data to support this point, and not a review article of Hedley fractionation (Yang and Post 2011).

Response: We changed the “P compounds” to “P fractions” to fulfill the sentence meaning. Yang and Post 2011 is actually a data compilation study, and therefore was maintained since it goes beyond any other individual study.

p. 10, lines 23-25: please cite an original study that contains data, and not a review paper (Huang et al. 2017) to support this statement of fact

Response: the phrase “quantitative data on the feedback between P compounds and biological communities during pedogenesis is still incipient to conclusions drawn from the influence of vegetation and organisms on the soil P composition (Huang et al. 2017)” is from the cited study, and it was originated because those authors had the same struggle as we had when evaluating other NMR studies, therefore it was maintained. There are several other instances in the manuscript where those specific responses were referenced from original NMR studies.

p. 11, line 6: “proportion” should be “proportions”.

Response: Alteration made.

p. 11, lines 26-28: These are also conditions under which ectomycorrhizal fungi are found. These fungi produce hyphal mats in the forest floor, so an increase in polyphosphates could reflect an increase in ectomycorrhizal hyphal mats.

Response: This explanation was added to the paragraph in the suggested place.

p. 12, line 5: “pH affect” should be “pH affects”

Response: Alteration made.

p. 12, line 5: “organic and inorganic P compounds” should be “Po and Pi compounds”

Response: Alteration made.

p. 12, line 14: “on the clay minerals”, delete “the”

Response: “the” was deleted.

p. 12, lines 15-18: I am pleased to see the authors citing original studies about DNA sorption at the start of this section. However, I do not understand why they cited Yu et al. 2013 at the end of the section (line 18), because this is a review paper. Please replace this citation with a paper containing original research to support this statement of fact.

Response: Cai et al. 2006, the original study, was cited instead of Yu et al. 2013.

p. 12, line 22: “for the P acquisition” delete “the”

Response: “the” was deleted.

p. 12, line 25: “organic P” should be “Po”

Response: Alteration made.

p. 12, line 28: “George et al. 2017” is a broad review paper; please cite a more specific reference to support this statement of fact.

Response: “George et al. 2017” was excluded and “Zimmerman et al. 2013” was maintained.

p. 12, line 28: “Plant and microorganism” should be “Plants and microorganisms”; “diesters” should be “orthophosphate diesters” or “phosphodiester”.

Response: Alterations were made through out the manuscript.

p. 12, line 29: “monoesters” should be “orthophosphate monoesters”, or “phosphomonoesters”; monoesters and diesters are general bond descriptions; the “orthophosphate” or “phospho” is more specific

Response: “orthophosphate” was added in all instances throughout the manuscript.

p. 12, line 30: Why is Turner 2008a cited to support this statement of fact about phosphatases? This was known years before the Turner paper was published (e.g. Halsted 1964, Skujins 1967, Tabatabai and Bremner 1969).

Response: All three references were cited to support the phosphatases statement.

p. 12, line 33: “both solubilization and hydrolysis by the phytase” As written, this implies that phytase both solubilizes and hydrolyzes inositol hexaphosphates, which is incorrect. Organic acids are required to desorb inositol phosphates, so that phytases can hydrolyze them. Please rewrite these lines, with a more appropriate reference than Turner 2008a.

Response: Turner 2008a reference was excluded for that state of fact.

p. 13, line 7: “effect in” should be “effect on”

Response: Alterations were made.

p. 13, line 8: “did not had” should be “had no”

Response: Alterations made.

p. 13, line 16: Huang et al. 2017 provides no direct evidence of phytate mineralization by ectomycorrhizal fungi. Please cite a better reference to support this statement of fact.

Response: Huang et al. 2017 was excluded Chen et al. 2004 was maintained.

p. 14, lines 12-13: Turner et al. 2002 is a review paper about inositol phosphates, so why is it being cited to support a general statement about

temperature and phosphatase activity? Please replace this with a more appropriate reference.

Response: Hui et al. 2013 was used instead of Turner et al. 2002.

p. 14, lines 24-29: Why are Walker and Syers 1976 and Feng et al. 2016 being cited to support discussion about decreased orthophosphate measured in NaOH-EDTA extracts by NMR? Neither of the cited references used NaOH-EDTA extraction or NMR, so they are irrelevant to the discussion here. Please replace these with better references. The authors should also mention here that caution needs to be used when discussing changes in orthophosphate extracted by NaOH-EDTA for NMR, because it will preferentially extract organic P rather than orthophosphate. As such, studies of the residuals after NaOH-EDTA show that it is mainly orthophosphate, especially for soils with low total P recovery by NaOH-EDTA.

Response: Walker and Syers 1976 and Feng et al. 2016 references were excluded from that specific sentence. Even though they measured orthophosphate with different methods, they refer to the same orthophosphate, and therefore one should expect that the mentioned responses of increased phosphorus losses due to increased precipitation won't change. Therefore, we left the other citations in the other parts of the paragraph. The caution sentence was added to the paragraph.

p. 15, line 9, "Even though" should be "However"

Response: Alteration made.

p. 15, lines 13 and 17: "Vitousek et al. 1995" is cited in the text but is not listed in the References

Response: Vitousek et al. 1995 was added to the references.

p. 15, lines 23-25: "phosphorus" should be abbreviated to be consistent with the rest of the manuscript.

Response: Alteration made.

p. 15, line 29: "why inositol hexakisphosphates have not been found" should be "why are inositol hexakisphosphates not found"

Response: Alteration made.

p. 16, line 15: “functional groups only (i.e. diesters and monoesters)” should be “broad compound classes only (i.e. orthophosphate diesters and monoesters”

Response: Alteration made.

p. 16, line 15: “when compounds concentrations” should be “when compound concentrations”

Response: Alteration made.

8. Conclusions:

p. 15, line 27: “as pedogenesis evolve” should be “as pedogenesis evolves”

Response: Alteration made.

9. Acknowledgements: “NSERC-Discover” should be “NSERC Discovery”; “from which studies” should be “from whose studies”

Response: Alterations made.

10. References: in general, this section is greatly improved compared to the previous version of this manuscript. However, there are still differences in formatting (e.g. Cloy et al. 2014) and some spelling mistakes (e.g. “Biophysical” in Wang et al. 2014”. Please proofread this section carefully.

Response: Cloy et al. 2014 was formatted. “Biophysical” was replaced by “Biophysical” in Wang et al. 2014. Several other minor details were formatted.

Environmental drivers of soil phosphorus composition in natural ecosystems

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Abstract. Soil organic and inorganic phosphorus (P) compounds can be influenced by distinctive environmental properties. This study aims to analyze soil P composition in natural ecosystems, relating organic (inositol hexakisphosphate, DNA and phosphonates) and inorganic (orthophosphate, polyphosphate and pyrophosphate) compounds with major temporal (weathering), edaphic and climatic characteristics. A dataset including 88 sites was assembled from published papers that determined soil P composition using one-dimensional liquid state ³¹P [nuclear magnetic resonance spectroscopy of NaOH-EDTA extracts](#), of soils. Bivariate and multivariate regression models were used to better understand the environmental properties influencing soil P. In bivariate relationships, trends for soil P compounds were similar for mineral and organic layers but with different slopes. Independent and combined effects of weathering, edaphic and climatic properties of ecosystems explained up to 78% (inositol hexakisphosphates) and 89% (orthophosphate) of [variations in organic and inorganic P compounds](#), across the ecosystems, likely deriving from parent material differences. Soil properties, particularly pH, total carbon and carbon-to-phosphorus ratios, over climate and weathering mainly explained the P variation. We conclude that edaphic and climatic drivers regulate key ecological processes that determine the soil P composition in natural ecosystems. These processes are related to the source of P inputs, primarily determined by the parent material and soil forming factors, [plant and microbe P cycling](#), the bio-physico-chemical properties governing soil phosphatase activity, soil solid surface specific reactivity and P losses through leaching, and finally the P persistence induced by the increasing complexity of [organic and inorganic P compounds](#) as the pedogenesis evolves. Soil organic and inorganic [P compounds](#) respond differently to combinations of environmental drivers, which likely indicates that each P compound has specific factors governing its presence in natural ecosystems.

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1 INTRODUCTION

Phosphorus (P) is a key nutrient in animal, microbial and plant nutrition and 'bears light' to terrestrial ecosystem functioning, regulating primary and secondary productivities (Walker and Adams 1958; Vitousek et al. 2010). Phosphorus input into a young ecosystem derives predominantly from the weathering of parent material, with some systems receiving P input from eolian deposits (Chadwick et al. 1999). Once P has been dissolved from primary minerals, plants and microorganisms access it from the soil solution. This P is then recycled through soil as organic and inorganic P (P_i and P_o , respectively) compounds (Noack et al. 2012; Damon et al. 2014), which are similarly subjected to a new cycle of physico-chemical and biological reactions. Each iteration of this cycle alters the form and bioavailability of the P, leading to decreasing levels of bioavailable P compounds (McDowell et al. 2007). In the absence of 'fresh' P inputs, this results in severe P limitations to ecosystem productivity (Walker and Syers 1976). The five state factors of soil formation (time, parent material, climate, topography and biota) determine the rate at which the cycle is completed (Jenny 1941). Therefore, a better understanding of the role of the five state factors as drivers of soil P composition is crucial to quantifying the relative abundance and form of both P_i and P_o pools.

In soils, P_i and P_o pools are each composed of specific P compounds (species) (Newman and Tate 1980; Tate and Newman 1982). The main P_o compound categories are: i) orthophosphate monoesters (single ester linkage to orthophosphate) such as inositol hexakisphosphates, ii) orthophosphate diesters (two ester linkages to orthophosphate) such as ribonucleic acid, deoxyribonucleic acid, lipoteichoic acid, phospholipid fatty acids, and iii) phosphonates. Inorganic P compounds include orthophosphate, polyphosphate and pyrophosphate. Specific phosphatase enzymes are required to transform the different P_o and P_i forms into orthophosphate, which is the P compound directly taken up by plants and microbes (Jackman and Black, 1952). As with most enzymes, the activities of soil phosphatases are very sensitive to the hydrogen potential (pH) with specific enzyme optima (Frankenberger and Johanson, 1982). However, other soil variables are also involved in regulating P_i and P_o transformations. For example, inositol hexakisphosphates bind strongly to metal oxides and other soil components, which strongly limits their bioavailability (Turner et al. 2007). Similarly, in pH lower than 5 (the isoelectric point of DNA), amino group protonation of adenine, guanine and cytosine bases in the DNA molecule can cause adsorption of positively charged DNA to the negatively charged clay surface (Cai et al. 2006). As a result, many soil properties regulate soil P composition but their relative importance across contexts is unclear.

The absolute and relative abundances of P_o and P_i forms and compounds are likely related to ecosystem development and soil weathering (McDowell et al. 2007; Turner et al. 2007), as conceptualized by the Walker and Syers model for P fractions (e.g., Walker and Syers 1976; Yang and Post 2011) (Figure 1, upper panel). As soils undergo pedogenesis, ecosystem productivity progresses from nitrogen (N) to P limitation with ecosystem productivity peaking at the N-P colimitation intermediate stage of pedogenesis (Walker and Syers 1976; Turner and Condron, 2013). Parallel changes occur in soil properties including a decrease in total exchangeable bases, soil acidification, and an increase in Al and Fe oxide concentration (Albrecht 1957; Walker 1965). As a result, some P_o and P_i compounds increasingly react with the

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mineral surface and progressively become occluded P (Yang and Post 2011). Subsequently, the complexity in P_o and P_i composition increases during ecosystem development (McDowell et al. 2007, Figure 1, bottom panel). The degree of soil weathering is inherently linked to the state factor 'time'—as demonstrated along many chronosequences (e.g. Turner and Laliberté 2015)—but it can be altered through other state factors (Albrecht 1957), such as along climosequences (e.g. Feng et al. 2016) or toposequences (e.g. Agbenin and Tiessen 1995). Along a climosequence, precipitation increased both base cation leaching and the degree of soil weathering, whereas potential evapotranspiration decreased these processes (Feng et al. 2016). While the Feng et al. (2016) study, evaluated the mineral-P associations described by the Hedley P fractions, rather than P speciation, it illustrates the opposing effects of various climatic factors on edaphic factors of interest.

The parent material has distinct effects on soil properties, all other state factors otherwise being equal. Some of these effects are direct effects, such as the total P concentration of the initial geologic material. However, other factors may be more indirect. Parent materials can differ in total exchangeable base concentration and mineral composition. Variations in mineral composition can lead to differences in soil pH, soil texture, and Al and Fe oxides, all of which influence soil P cycling and P composition. For instance, soil P retention potential is influenced by differential absorption of P_i and P_o to clays, soluble Ca content, as well as Al and Fe oxhydroxides (Batjes 2011). As such, the parent material state factor is an essential consideration in describing soil P cycling. Most importantly, we need to investigate the hierarchical nature of causal effects between state factors, soil weathering, soil properties, and P_o and P_i composition.

Nuclear magnetic resonance spectroscopy (NMR) is a widely-used method to study P_o and P_i compounds in ecosystems around the world (Kizewski et al. 2011). This technique can be used for both qualitative and quantitative estimates of P compounds in soil (Cade-Menun and Preston 1996). The most effective extractant for NMR analysis has been NaOH combined with the chelating agent EDTA (Cade-Menun and Liu 2014). This does not imply that NaOH-EDTA is the best extractant for ^{31}P NMR; however, because of its widespread use, it is a good baseline for comparison (Cade-Menun and Liu 2014). According to Cade-Menun and Preston (1996), NaOH can solubilize P_i and P_o compounds, while EDTA chelates metallic cations to increase P extraction efficiency from the soil. The NaOH-EDTA extraction method is widely recognized to quantitatively extract P compounds from the soil (Turner and Blackwell 2013, Cade-Menun and Liu 2014). However, there are drawbacks of using NaOH-EDTA extractant for ^{31}P NMR analysis. NaOH-EDTA does not extract all soil P and the highly alkaline environment can potentially degrade some P compounds (Cade-Menun et al. 2006; Cade-Menun and Liu 2014). Additionally, the high pH of the NaOH-EDTA extraction separates P species from the cations (e.g. Al, Fe, Ca) with which they were associated in soil, There are other methods to study P dynamics (Frossard et al. 2011), and soil P composition (Kruse et al. 2015), but none of these methods is perfect individually. For example, while X-ray absorption near edge structure (XANES), is a more preferred method for looking at orthophosphate speciation (Hesterberg 2010), and is a solid-state technique that does not require extraction, P concentrations are often below the detection limit. Therefore, XANES can only detect broad P species groups (e.g. Fe-P, Ca-P), but cannot, for example, determine if DNA is sorbed to Fe or Al. The most thorough studies of soil P use a combination of techniques together, and not any single technique (e.g. Liu et al. 2013; Liu et al. 2015).

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Moved (insertion) [1]

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Moved up [1]: There are many other methods available to study P dynamics (Frossard et al. 2011), and soil P composition (Kruse et al. 2015) in ecosystems.

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Moved up [2]: X-ray absorption near edge structure (XANES), is a more preferred method for looking at orthophosphate speciation (Hesterberg 2010).

There is a lack of broader understanding of how soil P composition is affected by different state factors of soil formation. Using a large-scale comparative geographical approach, we aim to determine the causal paths through which climate, parent material and time influence soil properties, as well as their impact on P_i and P_o pools and specific P compounds. Combining the soil P_i and P_o results obtained with ^{31}P NMR using NaOH-EDTA from different studies, allows us to describe the effect of state factors on soil P composition in natural ecosystems. We hypothesize that the compounds comprising soil P_i and P_o will be modified by distinctive edaphic and climatic properties due to different key ecological processes coupled with soil P cycling.

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2 METHODS

2.1 Dataset

A database search was conducted until November 17, 2017, to identify published papers that accurately determined soil P compounds through one-dimensional liquid state ^{31}P NMR on NaOH-EDTA extracts. According to McDowell et al. (2006) and Cade-Menun and Liu (2014), we consider as accurate the papers that used an adequate delay time prior to the NMR analysis, therefore enabling the production of quantitative data on the NMR instrument. We used two platforms and specific search terms for each one. The first platform was the Web of Knowledge. The following terms were used: "soil* phosphorus or P or ^{31}P * nuclear-magnetic-resonance or NMR* naoh or sodium hydroxide* edta or ethylenediaminetetraacetic" from which 129 results were obtained. The second platform was Google Scholar. The following terms were used: "soil* phosphorus* "nuclear magnetic resonance"* naoh* edta", which yielded 2,190 results (excluding patents and citations).

We followed pre-defined eligibility criteria to consider the papers, and then to select or reject them. The first criteria was that only native growth media were considered (manure, pot soil, soil leachate and sediment samples were excluded). In studies focusing on changing natural conditions, only the control (unchanged) samples were used, (e.g., litter removal in Vincent et al. 2010 was excluded). Next we only considered studies where the one-dimensional liquid state ^{31}P NMR method was used with the following features: 1) NaOH-EDTA extractor without pretreatment (0.5 or 0.25 M NaOH and 0.1 or 0.05 M EDTA), 2) delay times > 2 s (i.e., quantitative data, see Cade-Menun and Liu 2014); 3) NMR features or explanations according to ^{31}P NMR principles (see Cade-Menun and Liu 2014); and 4) total NaOH-EDTA extracted P and total P. Both top mineral and organic layers were considered. From selected papers, we compiled: total P, total NaOH-EDTA P, and NaOH-EDTA organic P, as well as the P_o compounds inositol hexakisphosphates (*myo*-, *scyllo*-, *neo*-, and *D-chiro*-IHP, when available), deoxyribonucleic acid (DNA), phosphonates, NaOH-EDTA inorganic P_i and the P_i compounds orthophosphate, pyrophosphate and polyphosphate. No duplicity was found in the selected papers.

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2.2 Site environmental properties

Soil texture, total C, total N and pH, short range ordered Al and Fe minerals (poorly crystalline) estimated with oxalate extraction, climate characteristics (mean annual precipitation and mean annual temperatures), as well as soil age, when

available, were also collected from the papers. When the total C was unavailable, the organic C was assumed to be the total C. This assumption only occurred for non-calcareous soils. Some variables were unavailable for some results, and the number of experimental used for each analysis is presented in the results section. Missing texture and total C data (representing 12 sites and one site, respectively) were extracted from a global soil dataset, SoilGrids, which is now at 250 m resolution (0-20 cm topsoil, Hengl et al. 2017). The resulting dataset is available in Appendix S1. We used the Whittaker's diagram (Whittaker 1975) and the "BIOMEplot" package (Kunstler 2014) to determine the biomes of our sites (Appendix S2).

Soil weathering stages were derived from the soil type according to Cross and Schlesinger (1995) and Yang and Post (2011) as well as from chronosequence positions. A low weathering stage was attributed to Entisol, Mollisols and Inceptisols forming the first stages of chronosequences and gleyed Acrisols. An intermediate weathering stage was attributed to Alfisol, Aridisol, Mollisols and Inceptisols forming the intermediate stages of chronosequences and orthic Acrisol. Finally, a high weathering stage was attributed to Oxisol, Spodosol, Ultisol, and humic Acrisol.

2.3 Data analysis

Statistical analyses were conducted on R Version 3.1.0 (© 2014 The R Foundation for Statistical Computing) using mixed-regression models including edaphic and climatic variables as continuous and categorical fixed effects. The latitude, the percentage of P extracted with NaOH-EDTA and the soil sampling depth were considered as random effects. Latitude was used to control for the spatial auto-correlation (Maestre et al 2005). The percentage of P extracted with NaOH-EDTA was used because the NaOH-EDTA extraction process varies according to soil characteristics and experimental conditions (i.e., pretreatment, soil-to-solution ratio and soil characteristics) (Cade-Menun and Liu 2014, see Fig. S5). Sampling depth was used because of potential differences in organic matter concentration along the soil profile. The bivariate effects of latitude, percentage of P extracted and sampling depth on the soil P composition are presented in Appendices S3-S5.

We used variation partitioning and Venn diagrams (Legendre and Legendre 2012) (the 'vegan' package) to partition the total variation explained uniquely by the matrix of either soil variables, climate variables or soil weathering stages as well as the variation explained by the combined effect of these matrices. The unique effect of soil, climate or soil weathering stages was calculated as the adjusted r^2 value (r_a^2) difference between the full model and unique model. The joint effect of these matrices was calculated as the difference between the summed r_a^2 of unique models and the r_a^2 of the full model.

Structural equation modeling (path analysis, the 'lavaan' package) was used to explore how variations in P_i and P_o compounds are driven by both direct and indirect effects of key environmental drivers (soil, climate and parent material). We first established an *a-priori* model that is based on our knowledge and is presented in Appendix 10. Then, we tested for the most parsimonious model among many alternative ones, i.e. the one that differed the least from the observations and presented the greater *P*-value. Parent material, which was unknown for our sites, was considered as a latent variable in the model to explain the remaining coordinated variations in total P, clay and pH variables that were not explained by climate and weathering. As mainly determined by the biota, total C was not considered as being constrained by the parent material.

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Different units were used across statistics to analyze soil P composition. The bivariate relationships (Figures 2, 3, and 5, and Appendices S3-S8) considered: i) total P_i or P_o concentration in NaOH-EDTA extracts (mg kg^{-1} soil), ii) proportion of total P_i or P_o as percentage of total NaOH-EDTA P (% of NaOH-EDTA P), and iii) proportion of soil P compounds as percentage of their respective pools (% of NaOH-EDTA P_i or P_o). In contrast, in both Venn diagrams (Figure 6) and structural equation modeling (Figure 7) soil P compounds were in mg kg^{-1} . In bivariate relationships, we used percentages to compare the relative composition of P along environmental variables that are linked with the weathering of soils. In both the Venn diagram and path analysis, the objective was to explain soil P composition either partitioning the variation among state factors or accounting for the causal structure of environment. For that, we used the mg kg^{-1} unit so that the distribution of our variables was not constrained as a proportion.

10 3 RESULTS

Our search resulted in 100 native vegetation outcomes from 13 references (Appendix S1) (Backnäs et al. 2012, n=1; Celi et al. 2013, n=4; Doolette et al. 2017, n=5; Li et al. 2015, n=1; McDowell and Stewart 2006, n=4; McDowell et al. 2007, n=26; Turner and Engelbrecht 2011, n=19; Turner et al. 2003, n=1, Turner et al. 2007, n=8; Turner 2008b, n=1; Turner et al. 2014, n=20; Vincent et al. 2013, n=8; Vincent et al. 2010, n=1). Most of the papers were excluded (from more than 2,000 papers found during the search) because they failed to meet the eligibility criteria including land use (e.g., crop, pasture, planted forest or wetlands) and ^{31}P NMR features. The results selected were from the following countries: Australia (n=5), Finland (n=1), Italy (n=1), New Zealand (n=59), Republic of Panama (n=21), Russia (n=4), Sweden (n=8) and the United States of America (n=1). These results comprised most of the global biomes classified according to Whittaker's diagram (Whittaker 1975), except for the subtropical desert, tundra and temperate rain forest. The six chronosequences studies (5 in New Zealand, 1 in Sweden; 5 on A layer, 2 on O layer) were the most important contributors to the data (45/74 sites on A layer, 18/20 sites on O layer).

In the compiled data, 80% of results were from mineral layers and the remaining 20% from organic layers; 39% did not contain inositol hexakisphosphates results (including all tropical regions), and 12% of DNA results were absent (including both non-tropical and tropical regions). The P extracted with NaOH-EDTA on mineral layers averaged 55% (2 to 98% range), and on organic layers averaged 73% (57 to 94% range) of total soil P. The average sample depth was 12.2 cm (2 to 42 cm range) for mineral layers and 11.0 cm (1 to 28 cm range) for organic layers.

3.1 Edaphic properties

All surveyed edaphic properties affected soil P_i and P_o pools and compounds. These results are summarized in Figures 2 and 3. Both total P_i (Figure 2A) and P_o (Figure 3A) concentrations in NaOH-EDTA extracts (mg kg^{-1} soil) had a quadratic response to soil pH, with higher values occurring at an intermediate pH, although this effect was constrained to the mineral layers. In mineral layers, the proportion of NaOH-EDTA P in the form of P_i increased with pH (Figure 2B), whereas the

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proportion in the form of P_{o_2} decreased with pH (Figure 3B). However, there was no pH effect on either pool in the organic layers. The distribution of compounds in both P_i (Figure 2C-E) and P_o (Figure 3C-D) pools responded dynamically to the pH. In the P_i pool (% of P_i) of mineral layers, orthophosphate decreased, and pyrophosphate accounted for the remaining P_i as the pH decreased. The pH had no effect on these P_o compounds in the organic layer (even though there is an apparent trend, these relationships became insignificant after including sampling depth as a random effect on models; Appendix S3 shows the sampling depth effect over the soil P composition). In the P_o pool (% of P_o), both inositol hexakisphosphates (mineral layer) and DNA (mineral and organic layers) proportions increased as the pH decreased. Phosphonates response to edaphic properties (insignificant) is presented in Appendix S6.

Both total P_i and P_o concentrations in NaOH-EDTA extracts (mg kg^{-1} soil) responded quadratically to the clay concentration, with higher values occurring at intermediate textural classes (Figures 2F and 3E). Clay impacted on neither P_i and P_o , nor on the proportions of P compounds (% of NaOH-EDTA P) (Figures 2G-J and 3F-H).

Total P_i and P_o concentrations in NaOH-EDTA extracts (mg kg^{-1} soil) increased as the soil C concentration increased in mineral layers, whereas in organic layers there was no C concentration effect on P_i and P_o concentrations (Figures 2K and 3I). As a percentage of NaOH-EDTA P, P_i decreased and P_o increased (% of NaOH-EDTA P) as the soil C concentration increased in mineral layers, and there was no C concentration effect on either P_i and P_o proportions in organic layers (Figures 2L and 3J). In the P_i pool (% of P_i) of mineral layers, orthophosphate and pyrophosphate proportions decreased and increased, respectively, as the soil C concentration increased (Figure 2M-O). As the soil C concentration increased in the organic layer, orthophosphate decreased, at a greater extent when compared to the mineral layer, pyrophosphate decreased (in contrast to the mineral layer, in which it increased), while the polyphosphate proportion increased, and gradually dominated the P_i pool at greater soil C concentrations. In the P_o pool (% of P_o), there was no C concentration effect on the soil P_o composition (phosphonates, inositol hexakisphosphates and DNA) in either mineral or organic layers (Figure 3K-L; Appendix S6).

Both total P_i and P_o concentrations in NaOH-EDTA extracts (mg kg^{-1} soil) from both mineral and organic layers increased as the total soil P concentration increased (Figures 2P and 3M). Only the DNA compound from the P_o pool (% of P_o) in the mineral layer was affected by the total soil P concentration (Figure 3P). As the total soil P concentration increased, the DNA proportion in the P_o pool decreased. It is important to note that the reported total P (x axis on Figures 2 and 3) is the one obtained by digestion and it includes both the extracted P and the residual P. The recovery of the total P by NaOH-EDTA extraction varies depending on soil characteristics and laboratory procedures (Cade-Menun and Liu 2014).

Total P_i and P_o concentrations in NaOH-EDTA extracts (mg kg^{-1} soil) were only affected by the soil CP ratio in organic layers. Both total P_i and P_o concentrations decreased as the soil CP ratio increased (Figures 2U and 3Q). As a percentage of NaOH-EDTA extract (% of NaOH-EDTA P), P_i decreased while P_o increased, both exponentially, as the soil CP ratio increased in mineral layers (Figures 2V and 3R). As proportions in the P_i pool (% of P_i) of mineral layers, orthophosphate decreased and pyrophosphate increased as the soil CP ratio increased (Figure 2W-X). In the P_i pool (% of P_i) of organic layers, proportions of orthophosphate decreased and polyphosphate increased, gradually dominating the P_i pool as

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the soil CP ratio increased (Figure 2W-Y). In the P_o pool (% of P_o), the DNA proportion increased as the soil CP ratio increased, only in the mineral layer (Figure 3T).

3.2 Climatic properties

5 | Climatic properties affected soil P_i and P_o pools and their composition only through the mean annual precipitation. These results are summarized in Appendices S7 and S8. The mean annual temperature, ranging from -0.4 to 27 °C, did not promote any change in the soil P composition in natural ecosystems. There was no effect of climatic variables on total P_i and P_o concentrations in NaOH-EDTA extracts (mg kg⁻¹ soil) (Appendices S7A and S8A). As a fraction of the NaOH-EDTA extract (% of NaOH-EDTA P), P_i decreased and P_o increased as the precipitation increased (Appendices S7B and S8B). As the precipitation increased, proportions of orthophosphate decreased and pyrophosphate increased as compounds of the P_i pool (10 | % of P_i) (Appendix S7C-D).

3.3 Soil weathering stages

Soil weathering stages determined from the soil type and chronosequence positions affected soil age and CP ratios following an expected effect of pedogenesis (Figure 4). As soil weathering stages increased, the soil age and CP ratio also increased. Both P_i and P_o pools were affected by the soil weathering stage (Figure 5). Total P_i and P_o in NaOH-EDTA extracts (mg kg⁻¹ 15 | soil) were more concentrated in soils at moderate weathering stages when compared to low and high weathering stages (n=79, Figure 5A, F). As percentages in the P_i pool (% of P_i), orthophosphate decreased and pyrophosphate increased as the soil weathering stage increased (n=79 for all P_y compounds, Figure 5C-D). In the P_o pool (% of P_o), the DNA (n=64) proportion was greater in more weathered stages, and there was no effect of weathering stages on phosphonates (n=79) and inositol hexakisphosphates (n=52) proportions (Figure 5H-J). Using available data (n=49), we observed no effect of soil 20 | weathering stages on short range ordered (poorly crystalline) Al and Fe minerals estimated with oxalate extraction (p>0.1, Appendix S9).

3.4 Variation partitioning among edaphic, climatic and weathering on the soil P composition

25 | The variation partitioning of ecosystem properties governing the soil P composition (in mg kg⁻¹ soil) was generally more pronounced for soil variables (pH, clay concentration and total P and C concentrations) than climatic variables (precipitation and temperature) and soil weathering (Figure 6). For the total P_i concentration and its compounds orthophosphate and pyrophosphate, the total variation explained by models ranged from 46% to 89%, and they were mostly explained by soil variables and combined effects of soil and weathering. Polyphosphates had a poorly-defined response to the variation partitioning of ecosystem properties (<0.01% of the total variation explained).

30 | In the P_o pool, the total variation explained by models ranged from 41 to 86% (Figure 6). The total P_o, inositol hexakisphosphates and DNA had their total variation mostly explained by soil variables, and to a lower degree, but more

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pronounced for the DNA compound, by combined effects of soil variables and weathering. In contrast, most of the variation in phosphonates was explained by combined effects of climate and soil variables, followed by uniquely soil variables.

3.5 Interdependences between environmental variables and soil P compounds

5 We used path analyses to explore the interdependences between edaphic and climatic variables and how they relate to the soil P_i and P_o compounds (Figure 7; Appendix S10). The parent material was used as a latent variable (set by the pH) in both models (P_i and P_o). Climate and soil weathering drivers were independently related to soil variables (total P, pH, clay and total soil C), and soil variables were considered direct effects in the models. The most parsimonious path analysis model explained up to 78% of P_o compounds variation and 89% of P_i compounds variation.

10 Following an expected effect of pedogenesis, the path analysis indicated that the parent material (latent variable) was positively related to the soil total P, clay and pH. Greater mean annual precipitation was negatively related to the soil total P, pH, and it positively influenced soil total C, while clay was negatively influenced by precipitation in the P_o model only. In the P_o model, precipitation promoted soil weathering, whereas in the P_i model, soil weathering was positively affected by temperature. The mean annual temperature positively affected the clay and pH. Soil weathering was negatively related to the soil pH, and positively related to the soil clay and total C. In the P_i model only, soil weathering negatively affected soil total P. There were also significant direct and positive effects between soil total C and clay, and total P, in both P_i and P_o models, and there was a positive relationship between soil total C and pH in the P_o model only.

15 In the P_i model, orthophosphate was negatively related to precipitation, and it was positively influenced by soil total P and total C. Pyrophosphate was positively influenced by precipitation, soil total P and total C. Polyphosphate was negatively influenced by temperature, and it was positively related to soil pH. In the P_o model, inositol hexakisphosphates were negatively affected by precipitation and temperature, but positively affected by the soil total P, total C and pH. In contrast, total P and total C positively affected DNA, and there were no effects of climatic variables over DNA. Phosphonates were negatively affected by temperature and weathering, but positively affected by precipitation and soil total C.

4 DISCUSSION

25 Our results showed how soil P_i and P_o compounds responded to edaphic variables (Figures 2 and 3), climatic variables (Appendices S7 and S8), and soil weathering stages as a proxy for pedogenesis (Figures 5) on a wide geographical scale, including a variety of natural ecosystems. While the soil P composition was primarily directly influenced by soil properties, the impact of climate and weathering stage occurred mainly through indirect paths and their influence on soil properties (Figures 6 and 7). In addition, soil P_i and P_o compounds responded to different combinations of explicative variables, which likely indicates that each P compound has specific factors governing its presence, transformation and persistence in ecosystems. This could be due to many factors including: i) the source of P inputs, primarily by minerals, and then altogether

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with plant and microbe P cycling; ii) the presence of specific phosphatase enzymes that are required to transform P_i and P_o compounds into orthophosphate; iii) the soil specific reactivity and P losses governed by physico-chemical properties (e.g., clay, short-range ordered oxides and pH); and iv) the P persistence induced by the increasing complexity of P_i and P_o compounds as pedogenesis evolves.

As time passes after the onset of pedogenesis, the ecosystem accumulates organic matter up to a maximum, and then starts to decline. Along with this decline, there are also changes in the chemical composition of organic matter, in which the decaying degree (i.e. decomposition) of C element is lower than the P, and concomitantly there is an increasingly acidic soil environment (Walker 1965). In addition, parent material supplies cations and orthophosphate to young soils, whereas more weathered soils are substantially changed from the parent material. Consequently, highly weathered soils generally have higher CP ratios, a lower pH and greater clay concentration. The soil total P content depends on both weathering stages and parent material, but generally decreases with increasingly weathered soil orders (Yang and Post 2011). Our data included soil orders ranging from all three stages of soil weathering (low, intermediate and high), according to Cross and Schlesinger (1995) and Yang and Post (2011). The soil weathering stage classification also takes into account changes in the soil P fractions, and generally follows the Walker and Syers (1976) conceptual model: there is gradual decrease and eventual depletion of primary mineral P (mainly apatite P), decrease of total P, increase and then decrease of total P_o and increase and eventual dominance of occluded P during the soil development (Yang and Post 2011). In highly weathered soils, occluded P increases through the encapsulation of the P_i and P_o compounds inside of Fe and Al minerals (McDowell et al. 2007; Turner et al. 2007).

Even though most results were from New Zealand and Panama, our dataset comprised several biomes according to the Whittaker's diagram (Whittaker 1975), including the temperate grassland desert, woodland shrubland, temperate forest, boreal forest, tropical rain forest, tropical forest savanna and intermediates between the temperate rain forest and boreal forest, and tropical rain forest and temperate rain forest (Appendix S2); however, quantitative data on the feedback between P compounds and biological communities during pedogenesis is still incipient to conclusions drawn from the influence of vegetation and organisms on the soil P composition (Huang et al. 2017), especially for ^{31}P NMR results. What is clearer is how soil P availability shapes the ecosystem's overall primary productivity, and to a lesser extent, soil food webs. In a global analysis, Maire et al. (2015) demonstrated that the soil available P is a key environmental dimension increasing leaf P content along with species' maximum photosynthetic rates and lower stomatal conductance. However, this trend is expected to gradually decline in more weathered soils due to a lower P availability. Conducted at a narrower scale, Laliberté et al.'s study (2017) showed that soil fertility (including P availability) strongly shaped underground food webs, promoting changes such as a shift in dominance from bacterial to fungal energy channels with increasing soil age.

4.1 Soil properties and the soil P composition

As soils aged, pyrophosphate and polyphosphate may have accumulated because of the incorporation and stabilization of these compounds (biological origin) into soil organic matter (Turner et al. 2007). The soil pH, total carbon and CP ratio, as

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well as weathering stage had a major influence on the soil P_i pool composition. As the orthophosphate proportion decreased in more weathered, acidic, organic-rich, and P-limited soil environments (Figures 2C, M, W and 5C), pyrophosphate and polyphosphate proportions increased and dominated the P_i pool (Figures 2D, N, O, X and Y and 5D).

Even though pyrophosphate and polyphosphate are P_i compounds, they have a biological origin (Turner and Engelbrecht 2011). Condensed forms of P (including pyrophosphate and polyphosphates) are found in every bacterial, archaeal and eukaryotic cell, but in highly variable amounts (Kornberg et al. 1999). Bünemann et al. (2008) found a positive relationship between the proportion of fungi and the amount of pyrophosphate, and Reitzel and Turner (2014) found a positive link between the pyrophosphate proportion and soil microbial P. Polyphosphate can originate from ectomycorrhizal fungi (Koukol et al. 2008), and there are some ectomycorrhizal fungi specialized for P uptake in low P, acidified soil conditions (Wang and Qiu 2006). Ectomycorrhizal fungi convert the orthophosphate that they take up from the soil into polyphosphates, and translocate the polyphosphate along fungal hyphae, sometimes at a great distance from where the orthophosphate is taken up (Bücking and Heyser 1999; Plassard and Dell 2010). Therefore, we believe that pyrophosphate and polyphosphate dominated the P_i pool in acidic, P-limiting (CP ratio), and high organic matter (total C) soils because of the microbial origin of these P sources, but much information is still needed in regard to plant and microbial communities characterization in studies of P forms. These organisms could have helped to deplete and transform the bioavailable orthophosphate, turning it into more microbial biomass derived P compounds as pedogenesis progressed in these environments.

Moreover, polyphosphates tend to occur in abundance only in soils where decomposition is slowed, such as acidified soil conditions, or cold and wet soils high in organic matter (e.g., Cade-Menun et al. 2000; Turner et al. 2004). Studying wetland soils, Cheesman et al. (2014) found that polyphosphates played a preeminent role in P-limited systems, predominantly in acidic, high-organic-matter systems. Adding to that, pyrophosphate hydrolysis was found to be more rapid with greater biological activity and higher agricultural soil pH (Sutton and Larsen 1964), and this may have contributed to reducing the pyrophosphate proportion at a higher pH in mineral soils (Figure 2D). As the C concentration increased in organic layers, polyphosphate dominated the soil P_i pool (Figure 2M-O) possibly because of its lesser lability when compared to orthophosphate and pyrophosphate. Pyrophosphate is less polymerized and potentially more susceptible to hydrolysis than polyphosphate. According to Savant and Racz (1972), Subbarao et al. (1977) and Dick (1985), pyrophosphate is hydrolyzed more rapidly than polyphosphate because pyrophosphate is an intermediate product of polyphosphate hydrolysis until the final orthophosphate is produced. These are also conditions under which ectomycorrhizal fungi are found. These fungi produce hyphal mats in the forest floor, so an increase in polyphosphates could reflect an increase in ectomycorrhizal hyphal mats. However, caution must be taken when interpreting pyrophosphate and polyphosphate hydrolyzation results from ^{31}P NMR analysis on NaOH-EDTA extracts. Polyphosphates can potentially degrade to pyrophosphates during extraction and NMR analysis of P (Cade-Menun et al. 2006), so they cannot be considered as fully distinct P forms. This potential degradation could be one explanation to why the polyphosphates results were poorly explained by the variation partitioning and structural equation models.

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As time passes after the onset of pedogenesis, modifications in the soil P_o composition were possibly related to the acidifying environment in soils, which may increase the charge of some P_o compounds, and thus increase sorption. Soil pH affects the sorption of P_o and P_o compounds by soils, but different P compounds respond differently to pH changes (Shang et al. 1992). Shang et al. (1992) verified that sorption of both orthophosphate and inositol hexakisphosphate by Al and Fe precipitates generally decreased as pH increased, whereas there was little pH effect on the adsorption of glucose 6-phosphate by both precipitates. Sorption of inositol hexakisphosphate to minerals surface is often stronger than orthophosphate, but both tend to be less sorbed to those minerals in neutral to alkaline soils (Berg and Joern 2006; Xu et al. 2017). The presence of humic acids may affect the amount of inositol hexakisphosphate that sorbs on the minerals surface at lower pH values, but it cannot displace inositol hexakisphosphate from that surface (Ruyter-Hooley et al. 2016). Moreover, another study finds that inositol hexakisphosphate sorption in soils was unaffected by the presence of orthophosphate, β -D-glucose-6-phosphate or adenosine 5'-triphosphate (Berg and Joern 2006). Following a similar pattern, the amount of DNA bound on clay minerals such as montmorillonite, kaolinite, hydroxyl aluminum species and variable charge soil colloids also increased by lowering pH of solution (Khanna and Stotzky, 1992; Cai et al. 2006; Cai et al. 2008; Wang et al. 2009; Saeki et al. 2010). At pH < 5, protonation of the amino groups of adenine, guanine and cytosine occurs and causes the increase of net positive charge of DNA and electrostatic attraction between negatively charged tetrahedral silica layer on the clay surface and DNA (Cai et al. 2006). Therefore, the increasingly acidic pH could have increased sorption, and therefore facilitated inositol hexakisphosphates (Figures 3C) and DNA (Figure 3D, Figure 7) accumulation in those soils.

In our study, we found that there was an increasing proportion of inositol hexakisphosphates and DNA in the P_o pool (% of NaOH-EDTA P) as pH decreased, and there was predominance of inositol hexakisphosphates in acidic, more weathered soils (Figures 3C-D, 5J). The hierarchy of investment for P acquisition through enzymatic activity may also be a factor that contributed to modifications in the soil P_o composition as time passes since the onset of pedogenesis. According to Turner et al. (2018), Turner and Haygarth (2005) and Kunito et al. (2012), P limitation may stimulate increased phosphoesterase synthesis as a way to increase bioavailable P by the mineralization of P_o . Fungi are well known for their capacity to secrete acid phosphatases (Rosling et al. 2016), and are usually the predominant microorganisms in acidic natural soils; while alkaline phosphatase and phytase genes are distributed across a broad phylogenetic range and display a high level of microdiversity (Zimmerman et al. 2013). Plants and microorganisms that breakdown orthophosphate diesters need a higher investment for the P acquisition (see Turner 2008a for the conceptual model) than orthophosphate monoesters, since they require hydrolysis by both phosphodiesterase and phosphomonoesterase to release available phosphate, whereas orthophosphate monoesters require only the last one (Halsted 1964; Skuijns 1967; Tabatabai and Bremner 1969). Although inositol hexakisphosphates are also classified as part of orthophosphate monoesters, they need a higher investment in P_o acquisition than other orthophosphate monoesters and diesters because they can be strongly bounded by metal oxides, clays and organic matter (Turner et al. 2007). Organic acids are required to desorb inositol phosphates, so that phytases can hydrolyze them, to release a free orthophosphate. This would suggest that as soil gets more weathered, inositol hexakisphosphates may accumulate more than DNA, and the latter more than other P_o compounds.

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Nonetheless, inositol hexakisphosphates can persist as a main P_o compound up to a certain point of pedogenesis, and then decline in more weathered soils. Some authors described that inositol hexakisphosphates declined to lower concentrations in older soils of non-tropical regions (Turner et al. 2014; Turner et al. 2007), and most tropical soils had negligible inositol hexakisphosphates contribution (e.g., Turner and Engelbrecht 2011). In our results, soil weathering had no effect on inositol hexakisphosphates concentrations in $mg\ kg^{-1}$ in non-tropical environments (Figure 7), but in fact, all compiled results from tropical soils had no inositol hexakisphosphates. Inositol hexakisphosphates have been found in very weathered soils (e.g., Oxisols), but under agricultural management that included well-known sources for that P compound such as plant seeds (Turner 2006; Smernik and Dougherty 2007; Deiss et al. 2016). These results suggest that inositol hexakisphosphates could occur in tropical soils under native vegetation, but they are either being rapidly turned into bioavailable compounds (by plants and microorganisms) or inputs of inositol hexakisphosphates, which are abundant in seeds and pollen (Raboy 2007), are lower in lowland tropical forests compared to temperate ecosystems (Turner and Engelbrecht 2011). In P limited soil environments, the acquisition of inositol hexakisphosphates may be strongly improved by root exudates, which may increase the solubility of these compounds in soil (Gerke 2015). In addition, mineralization of myo-inositol hexakisphosphate by ectomycorrhizal fungi (Chen et al. 2004) may also have contributed to its decline in more weathered, acidic soils, due to fungi predominance in these environments. Finally we believe that other P compounds such as DNA (Figures 3D, 5J) and pyrophosphate (Figures 2D, 5D) will possibly prevail in more weathered systems from tropical regions because they are intrinsic components of the microbial biomass (Kornberg et al. 1999). Mature soils are known for having the microbial P as the main component of the P pool (Turner et al. 2013).

Soil clay concentration affected both soil total P_o and P_i concentrations (Figures 2F and 3E), but had a minor association with soil P compounds. Recent investigations have found that organic compounds stabilization may be mainly driven by factors that are typically minor constituents of the clay-sized fraction by mass, but highly reactive components. Vogel et al. (2014) showed that organic matter is preferentially stabilized in certain hot-spot zones (i.e., rough surfaces), and that only a limited portion of clay-sized surfaces contributed to soil organic matter stabilization. This concept was further tested for P by Werner et al. (2017) who found that microscale spatial heterogeneity influences P accessibility and bioavailability in soil aggregates, depending on soil substrate and depth. They also found that in P-rich areas of soil aggregates, the P was predominantly co-located with Al and Fe oxides, while in low-P topsoil aggregates, most of the P was organically bound (Werner et al. 2017). Yang et al. (2016) also showed that only limited portions of fine mineral surfaces contributed to soil organic matter stabilization. So, these facts could justify the minor association between bulk soil clay concentration and soil P compounds as observed in our study.

Soil organic matter stabilization could be facilitated in more weathered soils by the potential increase in amorphous Al and Fe oxides (Albrecht 1957; Walker 1965), and consequently more reactive surface area availability to absorb and stabilize soil organic matter; however, we did not observe a significant overall soil weathering effect on Al and Fe oxide concentrations (Appendix S9), suggesting that it may depend on other factors such as the parent material or specific soil orders. Moreover, contrasting soil organic matter responses to short-range-ordered (amorphous) Fe or Al oxides have been

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found in literature. Some investigations found a pronounced role promoted by Al oxides (Heister 2016; Kaiser et al. 2016), whereas others found Fe oxides as the main soil organic matter stabilizing mechanism (Wilson et al. 2013; Catoni et al. 2016; Deiss et al. 2017). Investigations also found no apparent relationship between soil organic matter and both Al and Fe oxides (Cloy et al. 2014; Vogel et al. 2015; Rumpel et al. 2015). Therefore, we could not confirm the role of Al and Fe oxides as influenced by soil weathering stages over the soil P composition.

4.2 Climate and the soil P composition

Climatic variables exerted an important role on the soil P composition but to a lesser extent when compared to soil variables (Figure 6). Contradicting what we expected, our results showed that temperatures ranging from -0.4 to 27 C° had no effect on both P_i and P_o pools and their compounds (Appendices S7 and S8). It was expected that the soil P_o concentration would decrease with increasing temperatures because higher temperatures are optimal for the breakdown of the soil P_o compounds by the microbial biomass through phosphatase enzymes release (Hui et al. 2013). Hui et al. 2013 confirmed that greater maximum phosphatase activity occurred at incubation temperatures >25°C when compared to 20°C, but no differences were observed among temperatures greater than 25°C (Hui et al. 2013). Therefore, phosphatase activity may depend on the range and magnitude of temperatures; and our results covered a greater range of markedly lower temperatures, which may reduce microbial activity variability even more due to a slowdown in the microorganisms' metabolism.

In contrast, precipitation affected several variables in the soil P_i pool. This result was also expected based on the classic paper of Walker and Syers (1976), which suggested that pedogenesis depends predominantly on the volume of water leached through soil. In our results, the soil total P concentration, pH (Figure 7), and orthophosphate proportion (Appendix S7C) were negatively related to precipitation. As precipitation increased (Appendix 7C) and the soil was in a higher weathering stage (Figure 5C), the orthophosphate proportion (% of NaOH-EDTA P) possibly decreased because of increased leaching. However, caution needs to be used when discussing changes in orthophosphate extracted by NaOH-EDTA for ³¹P NMR, because NaOH-EDTA will preferentially extract P_o rather than orthophosphate. As such, studies that analyzed the residual P after NaOH-EDTA extraction have shown that it is mainly composed by orthophosphate (e.g. Cade-Menun et al 2005). Feng et al. (2016) evaluated P fractions along a climosequence and observed that greater precipitation (in soils with no impeded drainage) reduced the inorganic concentration of P linked to Ca, corresponding to a marked decline in soil exchangeable Ca and suggesting an enhanced leaching of P along with weatherable cations. Moreover, greater soil water availability, and consequent greater primary productivity, may have increased the demand for P in its bioavailable form and contributed to the orthophosphate depletion.

As the orthophosphate percentage and concentration (Appendix 7C and Figure 7, respectively) decreased following greater precipitation, the pyrophosphate percentage and concentration (Appendix 7D and Figure 7, respectively) increased suggesting that this compound predominates under these environmental conditions. As previously described, this may be due to the incorporation of these compounds into recalcitrant soil organic matter (Turner et al. 2007). Moreover, given the microbial origin of pyrophosphate and its association with the microbial P biomass (Koukol et al. 2008; Turner and

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Engelbrecht 2011; Reitzel and Turner 2014), pyrophosphate (Figure 7, Appendix 7D) possibly mirrored the response of the total P_o (Appendix 8B) to climatic variables, which may have resulted from greater soil organic matter accumulation following greater productivity (i.e., plants and organisms) in these ecosystems with greater water availability. Evaluating the P budget of the whole ecosystem, Turner et al. (2013) demonstrated the dominance of microbial P in mature soils. Wang et al. (2014) found that greater P_o concentrations were associated with increasing biomass production (i.e., primary production and microbial biomass) because plants and microbes incorporate P into biomass and return it to the soil. However, it is important to note that the majority of P in plant biomass is as orthophosphate (e.g. Noack et al. 2012) and not as P_o compounds. However, we believe that with higher orthophosphate inputs through plant biomass, soil P_o concentrations would increase altogether with orthophosphate P concentrations, and also at expense of soil orthophosphate due to the greater bioavailability to plants and organisms of that latter P compound.

Changes in vegetation are expected to occur during pedogenesis, and climatic variables may govern magnitudes of these alterations along with soil changes. Vitousek et al. (1995) showed that as ecosystems develop, the pattern of P concentration in plants leaves follows a non-linear response to time, in which lower concentrations occur at either early or late stages of pedogenesis, and a maximum is reached at an intermediate stage of pedogenesis. In addition, precipitation can affect the magnitude of that maximum response (intermediate stage), where the P concentration in plant leaves is higher in mesic gradients when compared to more wet gradients (Vitousek et al. 1995). Moreover, as described earlier, the soil available P, along with other climatic variables, governs maximum photosynthetic rates, but a trend that is expected to gradually decline in more weathered soils, due to a lower P availability (Maire et al. 2015). Phosphorus limitation can become sufficiently intense in the late stages of ecosystem development (also known as the retrogressive phase) to cause a decline in forest biomass, and productivity (Wardle et al. 2004). The exception seems to be tropical forests (Turner et al. 2007), which exhibit very diverse tree communities on old, infertile soils (Losos and Leigh 2004). Moreover, Turner et al. (2018) showed that in lowland tropical ecosystems, P limitation affects individual species, but species-specific P limitation does not translate into a community-wide response, because some species grow rapidly on infertile soils despite extremely low P availability.

4.3 Future research priorities

Many efforts have been made to explain soil P composition during pedogenesis; however, a clear picture on how specific plant species, plant functional traits, and their communities can influence the soil P composition is still lacking, especially with results obtained with ^{31}P NMR. For example, why are inositol hexakisphosphates not found in tropical soils under native vegetation, i.e., is it because the rapid turnover promoted by plants and, or organisms (which one?), or exclusively due to lack of inputs from plants? Does the changes in forest biomass and plant species diversity as soil P turns scarcer contribute to soil P composition in non-tropical environments, either by inputs or P compounds consumption, or the soil *per se* governs both the soil P composition and vegetation dynamics? Therefore, we point out that studies aiming to disentangle

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confounding effects among soil biotic and abiotic components, climate and vegetation are required to enable a better understanding of soil P composition in natural ecosystems.

Moreover, studies coupling ^{31}P NMR with other important techniques (e.g. Liu et al. 2013; Liu et al. 2015) could contribute to a better understanding of P cycling and composition in terrestrial ecosystems. A clear understanding of how orthophosphate species such as Fe-, Al- and Ca-phosphate (Hesterberg 2010; Kizewski et al. 2011) respond to pedogenesis could be elucidated with XANES (see Prietzel et al. 2013; Hashimoto and Watanabe 2014). Studies that quantify specific-P-related enzymes activity (see Turner et al. 2018) in native vegetation soils could help understand if the hierarchy of investment for the P acquisition actually contributes to different degrees of accumulation of inositol hexakisphosphates and DNA as pedogenesis progresses in non-tropical environments, and if phosphatases are leading to a rapid turnover of inositol hexakisphosphates in tropical environments. This can be achieved through determining the presence and abundance of microorganisms and enzymes, and how these changes affect soil P composition. Turnover, exchange kinetics, mineralization rates could be assessed using isotopic techniques (see Frossard et al. 2011), and enable a separation between different sources of P compounds, and their dynamics in soils, organisms and plants.

Finally, we expect future research to provide results of as many soil P compounds as they can find rather than broad compound classes only (i.e. orthophosphate diesters and monoesters), even when compound concentrations are low (and describe when main soil compounds are not detected), which may enable future analyses to avoid possible confounding effects of P compounds inside functional groups (e.g., inositol hexakisphosphates and orthophosphate monoesters) and to make a more precise correction for potential degraded peaks occurring during the alkaline extraction and reading process. We also urge researchers to determine variances or standard errors for soils with distinctive properties. Then, as stated by Stewart (2010), future analyses could use the different information provided by studies of different scopes and quality in a meta-analytical approach.

5 CONCLUSION

We conclude that edaphic and climatic properties are important factors in determining soil P_i and P_o pools as well as their compounds, since they regulate key ecological processes governing their presence, transformation and persistence in soils. These processes are related to the source of P inputs, primarily determined by parent material and then altogether through plant and microbe P cycling, the bio-physico-chemical properties governing soil phosphatase activity, soil solid surface specific reactivity and P losses through leaching, and finally the P persistence induced by increasing complexity of P_i and P_o compounds as pedogenesis evolves. Soil drivers that played a preeminent role were soil acidification, C concentration, P limitation determined as CP ratio, soil weathering as the temporal variable, while precipitation was the climatic variables that most influenced soil P composition. Soil P composition was more influenced by soil variables than either climatic variables or weathering stages. However, combined effects among these factors also contributed to explain considerable soil P variability in these ecosystems. Soil P_i and P_o compounds responded differently to combinations of environmental drivers,

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which likely indicates that each P compound has specific factors governing its existence in natural ecosystems. Therefore, knowing how environmental drivers affect soil P composition enabled a comprehensive understanding of soil P in natural ecosystems.

6 APPENDICES (SUPPLEMENTARY FILES)

Appendix S1 – Dataset: The dataset is attached as a supplementary Excel file.

Appendix S2 – Global biomes comprised in our dataset according to the Whittaker' diagram.

Appendix S3 – Soil depth effect on soil P composition.

5 **Appendix S4** – Latitude effect on soil P composition.

Appendix S5 – Percentage of P extracted with NaOH-EDTA effect on soil P composition.

Appendix S6 – Soil properties and soil organic phosphonates.

Appendix S7 – Climatic properties and soil inorganic phosphorus.

Appendix S8 – Climatic properties and soil organic phosphorus.

10 **Appendix S9** – Soil weathering stages and poorly crystalline Al and Fe concentration.

Appendix S10 – Models tested to explore the interdependences between edaphic and climatic variables (path analysis) as the main environmental predictors of soil inorganic and organic P compounds.

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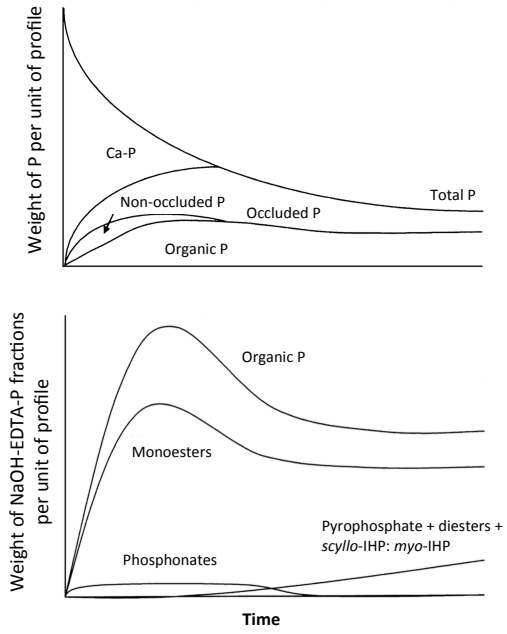
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5 Figure 1: Conceptual diagrams of the changes in soil P fractions (above) and NaOH-EDTA extractable P compounds (excluding orthophosphate) (below) with time (redrawn from Walker and Syers, 1976, with permission from Elsevier, and McDowell et al. 2007, with permission from John Wiley and Sons, respectively).

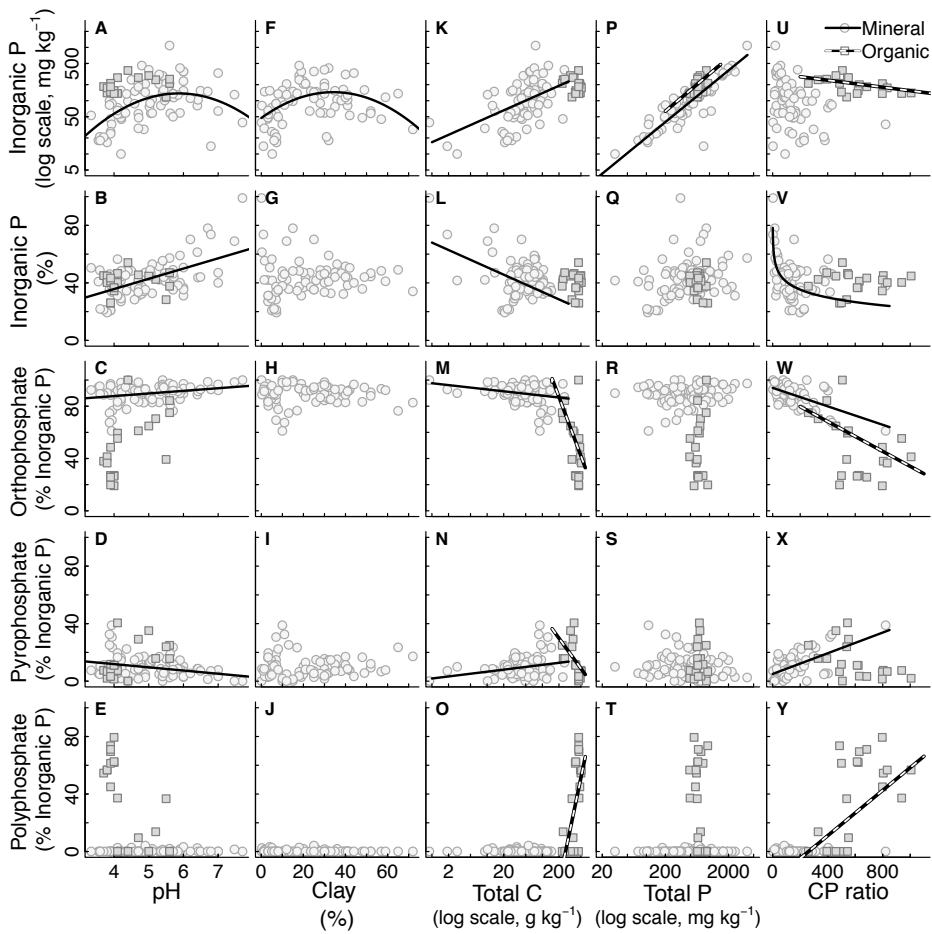


Figure 2: Relationship between edaphic properties and soil inorganic phosphorus (P) composition in NaOH-EDTA extract from soil mineral and organic layers on natural ecosystems. Note that the reported total P is the one obtained by digestion and usually comprise the residual P non-recovered by the NaOH-EDTA extractant. Regression models (n = 80 mineral layer and n = 20 organic layer): mineral layer, $\log(\text{total P}_i \text{ mg kg}^{-1}) = -1.62 + 1.28 \text{ pH} - 0.11 \text{ pH}^2$, $r^2 = 0.33$; mineral layer, $\text{total P}_i (\%) = 7.21 + 7.12 \text{ pH}$, $r^2 = 0.34$; mineral layer, $\text{orthophosphate} = 79.7 + 2.00 \text{ pH}$, $r^2 = 0.11$; mineral layer, $\text{pyrophosphate} = 20.8 - 2.23 \text{ pH}$, $r^2 = 0.11$; mineral layer, $\log(\text{total P}_i \text{ mg kg}^{-1}) = 1.68 + 0.028 - 0.00041 \text{ clay}^2$, $r^2 = 0.23$; mineral layer, $\log(\text{total P}_i \text{ mg kg}^{-1}) = 1.22 + 0.46 \log(\text{total C})$, $r^2 = 0.32$; mineral layer, $\text{total P}_i (\%) = 68.0 - 17.2 \log(\text{total C})$, $r^2 = 0.14$; mineral layer, $\text{orthophosphate} = 97.6 - 4.74 \log(\text{total C})$, $r^2 = 0.08$; organic layer, $\text{orthophosphate} = 348.0 - 113.4 \log(\text{total C})$, $r^2 = 0.30$; mineral layer, $\text{pyrophosphate} = 1.85 + 4.74 \log(\text{total C})$, $r^2 = 0.08$; organic layer, $\text{pyrophosphate} = 151.7 - 53.0 * \log(\text{total C})$, $r^2 = 0.34$; organic layer, $\text{polyphosphate} = -446.4 + 184.4 \log(\text{total C})$, $r^2 = 0.45$; mineral layer, $\log(\text{total P}_i \text{ mg kg}^{-1}) = -0.63 + 0.97 \log(\text{total P})$, $r^2 = 0.73$; organic layer, $\log(\text{total P}_i \text{ mg kg}^{-1}) = -0.63 + 0.97 \log(\text{total P})$, $r^2 = 0.73$.

$P_i \text{ mg kg}^{-1} = -0.50 + 1.00 \log(\text{total P})$, $r^2 = 0.27$; organic layer, $\log(\text{total } P_i \text{ mg kg}^{-1}) = 2.51 - 0.00033 \text{ CP ratio}$, $r^2 = 0.26$; mineral layer, $\text{total } P_i (\%) = 77.0 - 7.88 * \log(\text{CP ratio})$, $r^2 = 0.33$; mineral layer, orthophosphate = $94.0 - 0.0353 \text{ CP ratio}$, $r^2 = 0.37$; organic layer, orthophosphate = $91.1 - 0.00057 \text{ CP ratio}$, $r^2 = 0.19$; mineral layer, pyrophosphate = $5.04 + 0.0359 \text{ CP ratio}$, $r^2 = 0.37$; organic layer, polyphosphate = $-20.5 + 0.079 \text{ CP ratio}$, $r^2 = 0.31$.

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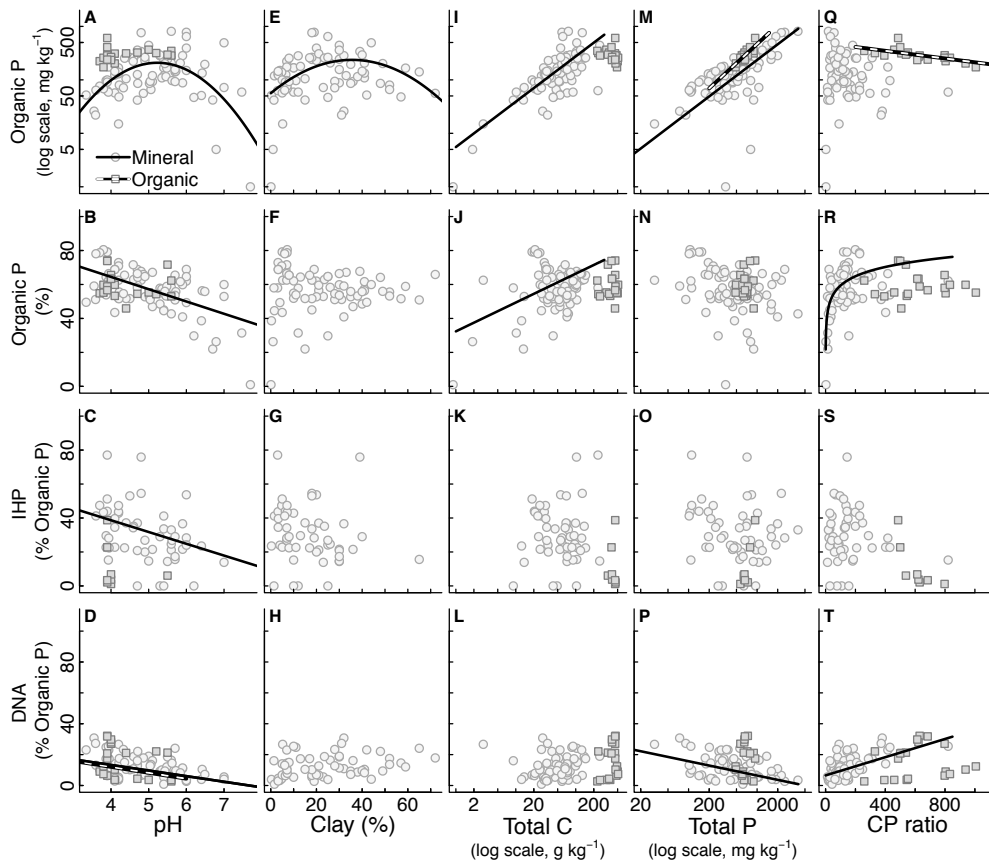
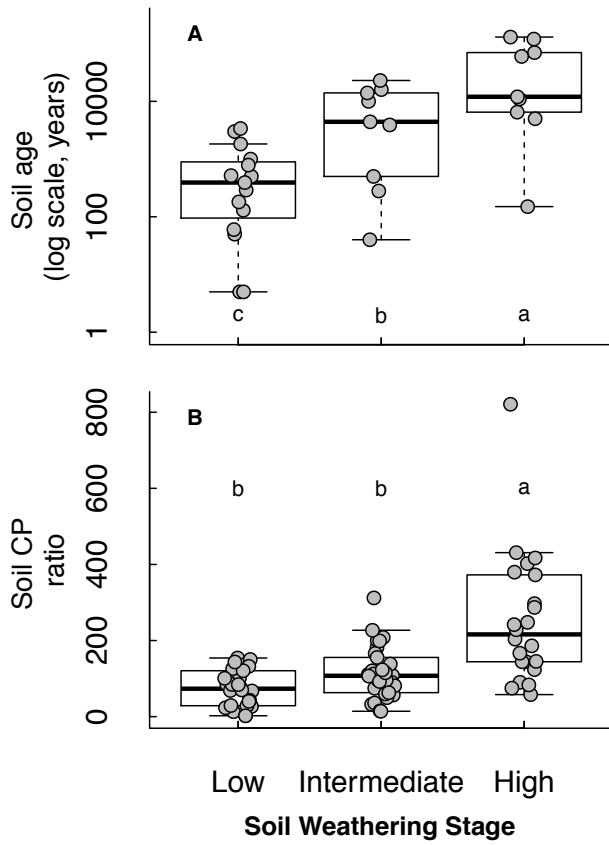


Figure 3: Relationship between edaphic properties and soil organic phosphorus (P) composition in NaOH-EDTA extract from soil mineral and organic layers on natural ecosystems. Note that the reported total P (x axis) is the one obtained by digestion and usually comprise the residual P non-recovered by the NaOH-EDTA extractant. Regression models: mineral layer, $\log(\text{total P}_o, \text{mg kg}^{-1}) = -3.61 + 2.27 \text{ pH} - 0.22 \text{ pH}^2, r^2 = 0.34$ (n=80); mineral layer, $\text{total P}_o (\%) = 93.4 - 7.24 \text{ pH}, r^2 = 0.35$ (n=80); mineral layer, inositol hexakisphosphate (IHP) = $66.4 - 6.94 \text{ pH}, r^2 = 0.16$ (n=52); mineral layer, DNA = $27.8 - 3.63 \text{ pH}, r^2 = 0.19$ (n=64); organic layer, DNA = $27.4 - 3.83 \text{ pH}, r^2 = 0.10$ (n=20); mineral layer, $\log(\text{total P}_o, \text{mg kg}^{-1}) = 1.75 + 0.035 \text{ clay} - 0.00049 \text{ clay}^2, r^2 = 0.16$ (n=80); mineral layer, $\log(\text{total P}_o, \text{mg kg}^{-1}) = 31.2 + 120.5 \log(\text{total C}), r^2 = 0.60$ (n=80); mineral layer, $\text{total P}_o (\%) = 32.4 + 17.0 \log(\text{total C}), r^2 = 0.12$ (n=80); mineral layer, $\log(\text{total P}_o, \text{mg kg}^{-1}) = -0.55 + 0.97 \log(\text{total P}), r^2 = 0.29$ (n=80); organic layer, $\log(\text{total P}_o, \text{mg kg}^{-1}) = -0.89 + 1.19 \log(\text{total P}), r^2 = 0.68$ (n=20); mineral layer, DNA = $34.2 - 9.27 \log(\text{total P}), r^2 = 0.18$ (n=64); organic layer, $\log(\text{total P}_o, \text{mg kg}^{-1}) = 2.69 - 3.58 \cdot 10^{-4} \text{ CP ratio}, r^2 = 0.48$ (n=20); mineral layer, $\text{total P}_o (\%) = 23.0 + 7.90 * \log(\text{CP ratio}), r^2 = 0.33$ (n=80); mineral layer, DNA = $6.53 + 0.029 \text{ CP ratio}, r^2 = 0.34$ (n=64).



5 Figure 4: Soil weathering stage relationship with soil age (n = 33) and CP ratio (n = 78) on natural ecosystems.

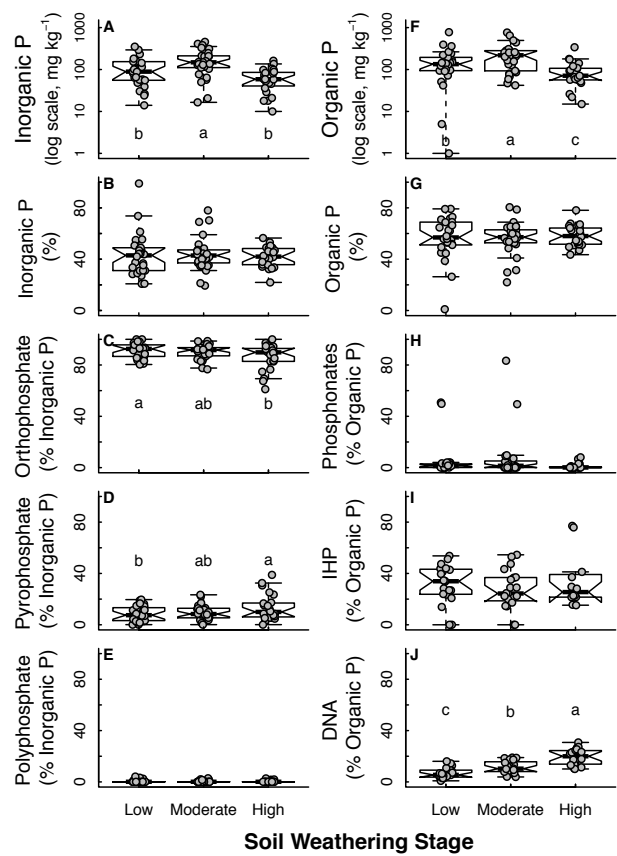


Figure 5: Soil inorganic and organic phosphorus (P) composition in NaOH-EDTA extract as influenced by weathering stages on natural ecosystems.

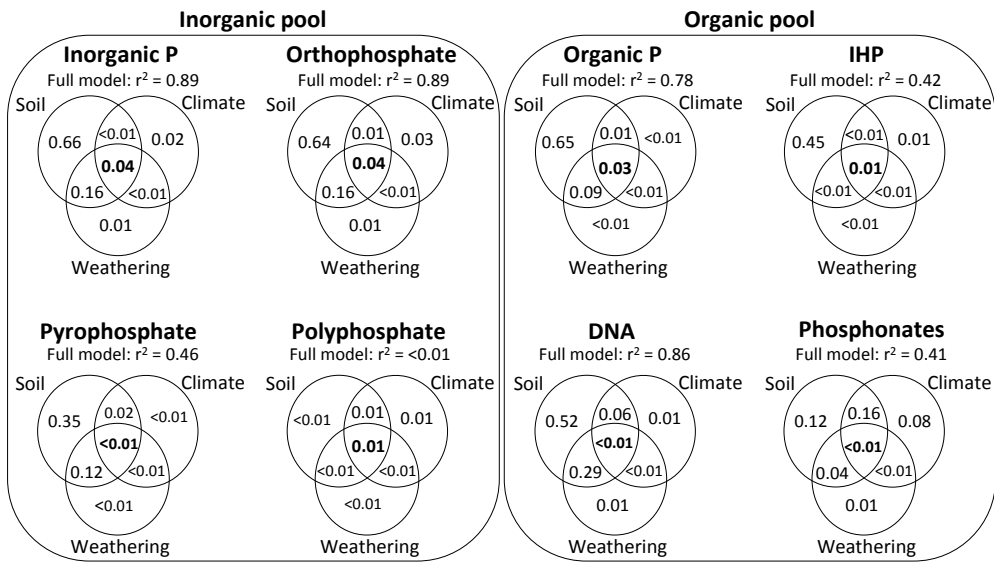
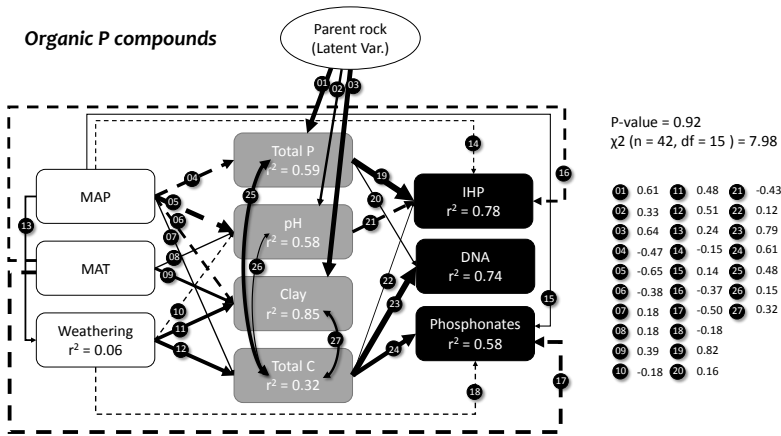
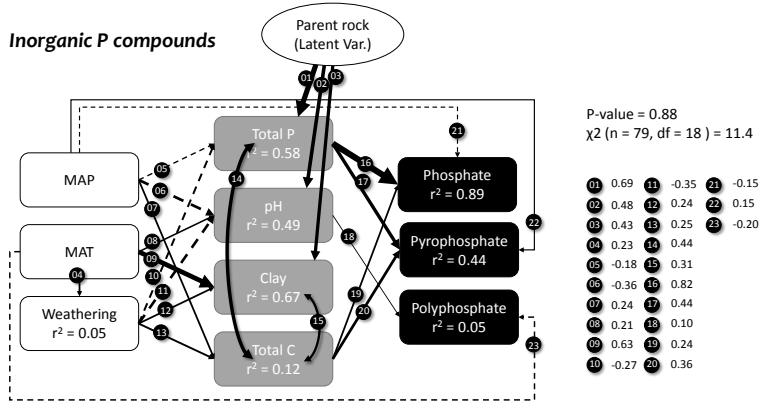


Figure 6: Variation partitioning among edaphic, climatic, and weathering stages on soil inorganic and organic P composition in NaOH-EDTA extract on natural ecosystems. Soil organic and inorganic P forms and compounds were in mg kg^{-1} , and the other variables followed units described on Figures 2 and 3.

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5 Figure 7: Path analysis describing the direct and indirect effects of the main environmental predictors of soil inorganic and organic P compounds (mg kg^{-1}) in NaOH-EDTA extract as influenced by edaphic and climatic drivers on natural ecosystems. Solid and dashed lines represent positive and negative relationships, respectively. Soil organic and inorganic P compounds were in mg kg^{-1} , and the other variables followed units described on Figures 2 and 3.

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<p>There are many other methods available to study P dynamics (Frossard et al. 2011), and soil P composition (Kruse et al. 2015) in ecosystems. X-ray absorption near edge structure (XANES), is a more preferred method for looking at orthophosphate speciation (Hesterberg 2010). This does not mean that the results on pyrophosphate, polyphosphates and total orthophosphate concentrations are not useful, however, there are other inorganic P compounds of importance in soils.</p>		
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