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Title: Pasture degradation decreases organic P content of tropical soils due to soil structural decline

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Corresponding Author: Dr. Astrid Oberson,

Corresponding Author's Institution: ETH Zurich

First Author: Maike Nesper

Order of Authors: Maike Nesper; Else K Bünemann; Steven J Fonte; Idupulapati M Rao; Jaime Velásquez; Bertha Ramirez; Django Hegglin; Emmanuel Frossard; Astrid Oberson

Abstract: Degradation of tropical pastures on highly weathered soils is linked to soil structural decline and associated losses of organic matter, which could both also affect soil phosphorus (P) dynamics and availability. Our aim was to elucidate these linkages by examining the effect of pasture degradation on the contents and forms of P contained within aggregate size classes and macroaggregate fractions (collectively referred to as soil structural components). We conducted a study on nine farms in a deforested part of the Colombian Amazonia, each farm with degraded and productive Brachiaria spp. pastures. Topsoil (0-10 cm) samples were separated by wet-sieving into aggregate size classes, and macroaggregates further separated into occluded fractions. Soils and structural components were analyzed for concentrations of total P, available P (extracted using anion exchange resins), NaOH-EDTA extractable organic and inorganic P, and enzyme-hydrolyzable organic P (simple monoester-like, DNA-like, myo-inositol hexakisphosphate-like) in the NaOH-EDTA extracts. Degraded pasture soils had significantly fewer large macroaggregates and more microaggregates, both with significantly lower organic P concentrations (in mg P kg-1 of structural component) than those in productive pasture soils. At the same time, total and extractable inorganic P concentrations in bulk soil and structural components did not differ between pasture types, suggesting a shift from organic to non-extractable P upon degradation. Soils under productive pastures contained 37% more organic P than degraded pasture soils (in mg P kg-1 soil), mainly in large macroaggregates. The organic P concentrations were strongly correlated with C concentrations across all soil structural components, suggesting similar stabilization mechanisms for organic P and C. In bulk soil and most structural components, around 60% of organic P was enzyme-hydrolyzable. The lower contents of all enzyme-hydrolyzable as well as enzyme-stable organic P in degraded vs. productive pasture soils indicate a reduction of all organic P classes during pasture degradation. The large macroaggregates and, in particular, microaggregates occluded within this fraction were identified as an important site for organic P storage. Our results highlight a linkage between soil structure and organic P, both of which can play an important role in maintaining the productivity of pastures established on highly weathered soils.

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Institute of Agricultural Sciences

Eschikon Experimental Station
CH-8315 Lindau
Switzerland

Dr. Astrid Oberson
Eschikon 33 P.O. 185
+41-52-354 9132
+41-52-354 9119
astrid.oberson@usys.ethz.ch
http://www.plantnutrition.ethz.ch/

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Dear Ben

Many thanks for acceptance of our paper under the condition of revisions, and to the reviewers for their constructive comments which helped us to improve our manuscript. In the enclosed reply to the reviewers, we describe (with blue font) how we considered their suggestions. We additionally send two versions of the revised manuscript, one with tracked changes and the other with all the changes accepted.

Hoping that this paper can now definitely be accepted, we thank you for the work for our paper and for this special issue on *Soil organic P dynamics.*

In the name of the author team

Astrid

COMMENTS FROM EDITORS AND REVIEWERS

In blue font the reply of the authors to the reviewer comments, with line numbers refering to the version with the tracked changes.

Reviewer #1: This manuscript describes a study into the impact of pasture degradation, on highly weathered soils, on the availability of organic P and its relation to carbon content and soil physical conditions. The data demonstrates that there is a relationship between organic P storage and soil structure, with large macroaggregates and microaggregates being important sites of organic P storage. The data go on to demonstrate that pasture degradation leads to loss of organic C, organic P and macroaggregate structure. This research is of interest to the readers of Geoderma. The manuscript is a well-conceived and thorough study of the subject and presents some interesting and important findings. There are still a few minor issues with the presentation that are highlighted below, but I believe the manuscript could be accepted following minor revisions.

1. My major issue with the manuscript is that the discussion is too long and detailed given the data presented. It becomes a little repetitive and loses the point a little. There is a strong story here and a concise discussion will present this important data better. Section 4.3 is the real meat of the paper and this should take precedence. Section 4.4 goes beyond the scope of the data presented and could be reduced or removed completely. In contrast the Results section is very short given the amount of data presented and should perhaps be presented in a bit more detail. I think a thorough re-writing of these sections will improve the impact of this paper.
Thanks for the propositions, we agree and have shortened the discussion accordingly, section 4.4 was largely been removed (see lines: 528 - 534 and lines 553- 565). We made some amendments to the result sections after a careful checking that we pointed the reader to the main findings.
2. One of the main conclusions is that with pasture degradation there is a loss of organic P that leads to a reduction in the productivity of the system. Is this relationship causative or not?
Probably our first sentence in the conclusion of the previous version suggested this causality and we have revised it accordingly. However, we do not know if this relationship is causative and throughout the paper we do not claim that it is causative (see lines: 474 - 478 and lines 576 580: “Degraded and productive pasture soils and their structural components had similar total P concentrations. Thus, we have no indication that soil P depletion was causing pasture degradation. However, the supply of plant available P could be lower in degraded than productive pasture soils because of reduced biological P cycling and because of increased P sorption due to less aggregation.”
Could it also be that the breakdown of aggregate stability as a consequence of C mineralization leads to loss of organic P also? Perhaps the alternate explanation should be made also.
Yes, we agree and we discuss several explanation in lines 474 onwards: “Several mechanisms could contribute to lower organic P contents in degraded than productive pasture soils, which are i) reduced organic inputs, ii) less organic P synthesis due to lower microbial activity, iii) loss of organic P due to reduced physical protection of SOM in aggregates and iv) due to prolonged anoxic soil conditions. These mechanisms are interlinked, and are discussed in detail below.” The following detailed discussion is not all copied here, but please note that the aspect of C and organic P mineralization is discussed in lines 521 – 522 “The lower organic P contents at similar total P contents indicate that due to lower protection, this P was mineralized.” The linkage between C and organic P is additionally discussed in lines 352-362.
3. You see little difference in the enzyme labile P in the various physical fractions with NaOH-EDTA extraction. What if you used a more biologically relevant extract, e.g. citrate, would you still see no difference in biochemical availability? Should this be highlighted in the discussion?
Our aim was to characterize total soil organic P. This is why we used enzyme addition to NaOH-EDTA extracts. However, we agree that also application of milder extractants would be interesting, provided sufficient P would be extracted and that the increase in molybdate reactive P following the enzyme addition could be detected. We now discuss this option in the paper (lines 421 - 426): “Our aim was to characterize total organic P using NaOH-EDTA extraction in combination with enzyme

additions. The observed small differences in enzyme-hydrolyzable P between structural components do not preclude that differences in enzyme-hydrolyzable P would be seen in milder extracts, such as citrate (Hayes et al., 2000), water or bicarbonate (George et al., 2007). Such milder extractants can only be used in soils in which they extract sufficient P to observer an increase in molybdate-reactive P following enzyme addition."

4. Is averaging productive and degraded pasture across 9 sites appropriate? Are we missing some important dynamic that may be apparent between the different sites. Do all sites/soils behave the same way?

The farms of the study region were included in earlier studies of the Caqueta region (e.g., (Fonte et al., 2014a; Grimaldi et al., 2014) and belong to the belt around the city of Florencia with the same classical pasture systems. All farms have a similar land-use history in comparable landscape positions with similar vegetation and present use. We explain this in our materials and methods section, and more details are given in Fonte et al 2014. The clear differences between the productive and degraded pastures indicate that there is a common pattern among all sites and soils and that pasture type is an important variable in explaining overall variability observed across sites. Please also consider similar, published studies (e.g., (Asner et al., 2004)) that considered fewer sites.

5. There is a bit of confusion in the paragraph from line 189 to 213. You describe the enzyme assays in the order phytase, APase, DNAase and then describe the pools that are measured in a different order. To avoid confusion these should be in the same order and the enzyme used to derive each pool should perhaps be highlighted as well.

Thanks. We now changed the order in the presentation of the enzymes used. We also clarified the paragraph where the enzyme classified forms are described, including change of the font to Italic to indicate calculations. The entire section now reads (lines 201 – 224):

"The following enzymes were added to the NaOH-EDTA extracts in a microplate assay (Keller et al., 2012): (1) Acid phosphatase (EC 3.1.3.2) from potato, Sigma Chemicals, Product No.P1146, (2) Nuclease P1, from *Penicillium citrinum*, Sigma Chemicals, Product No.N8630 together with acid phosphatase, and (3) Phytase from *Peniophora lycii* (RONOZYME® NP (M), DSM Nutritional Products Denmark). (……..) Enzyme-hydrolyzable organic P was classified based on the enzyme specificity determinations of Annaheim et al. (2013)):

(1) Simple monoester-like organic P: P in simple monoesters like glycerol phosphate, glucose-6-phosphate, in anhydrides and in condensed organic compounds like pyrophosphate and ATP; *P released by acid phosphatase*.

(2) DNA-like organic P: P in nucleic acids; *(P released by nuclease plus acid phosphatase) – (P released by acid phosphatase)*.

(3) InosP-like organic P: P in *myo*-inositol hexakisphosphate; *(P released by phytase) – (P released by acid phosphatase)*."

6. Nomenclature for some of the fractions is inappropriate. E.g. clay&silt, this isn't a standard way of referencing this I would prefer clay/silt fraction. Also mM is very confusing, to many people this is synonymous with milli mole and just adds to confusion. I would change both of these.

Yes, we agree that mM can be confused with milli mole and have therefore changed the abbreviation for microaggregates within macroaggregates from mM to Mmicros in the text, tables and figures. Regarding the silt&clay we have evaluated other naming possibilities but concluded that this is the most adequate abbreviation for the fraction we want to describe since it expresses the combination of both size classes silt and clay. With the abbreviation "clay/silt" the reader might be confused by the divided sign. Also due to the "&" and the removal of the spaces between "silt" and "clay" it is clearly noticeable within the text that we refer to one combined fraction.

7. I think there are a number of key references missing. I don't consider some of those used as the definitive reference. For example there are a trail of references that have led to the enzyme labile methods which are not mentioned, Shand et al., Hayes et al., Richardson et al. Turner et al. and George et al.

Thank you for pointing this out. We have reworded the section slightly to include also earlier work and we have included other references in the discussion (see reply to point 3). The section in the introduction now reads (lines 73 – 87): "A single-step procedure involving sodium hydroxide and

EDTA (ethylenediaminetetraacetate) is often used for the extraction of soil organic P from strongly weathered tropical soil (Turner, 2008). Additions of enzymes to various soil extracts have been used to characterize hydrolyzable forms of organic P, as reviewed by Bünemann (2008), e.g., addition of acid phosphatase or phytase to soil water extracts (Fox and Comerford, 1992; Shand and Smith, 1997). As different enzymes hydrolyze different organic P bonds, this approach delivers information on the chemical form of organic P in soils (He and Honeycutt, 2001; Turner et al., 2002). Based on P release by acid phosphatase, phytase and nuclease from defined organic P compounds, Annaheim et al. (2013) classified the hydrolyzed P into simple monoesters, nucleic acids and *myo*-inositol hexakisphosphate. Keller et al. (2012) used the same approach to classify organic P in NaOH-EDTH extracts. Enzyme-hydrolyzable organic P identified by phytase addition to EDTA extracts was shown to be greater in Oxisols under no till than with conventional tillage (Pavinato et al., 2010), suggesting that enzyme-hydrolyzable organic P is a sensitive indicator for the impacts of soil management on soil P status."

8. There is reference to organic P being recycled on to the soil through pasture, animal to manure. How much of the P that goes through this cycle is actually organic. My understanding is that a lot of this is actually inorganic P, which is converted to organic by immobilization in microorganisms.

We agree that through animal manure mainly inorganic P is cycled back, but we consider that also the organic matter input via manure stimulates overall biological P cycling, and that the about 25% of organic manure P cannot be neglected. We have revised the section (line 479 - 483), which now reads: "The input of C and organic P contained in aboveground plant litter (Table 1) and in belowground residues (because of lower root density ((Fonte et al., 2014a)) was lower in degraded than in productive pastures. Furthermore, due to the lower amount of forage offered by degraded pastures, less C and organic P in animal manure were recycled back to the soil."

Please also consider that in line 487 we explain that most of the organic P is of microbial origin and throughout the section 484 – 490 the importance of microbial P synthesis is discussed.

9. Misuse of the word high and low throughout. These should only be used for height, temperature and rates and replaced with greater, heavier, longer etc.

In general we agree with the reviewer's comment, but note that in most cases the terms 'higher' and 'lower' are clearly associated with a rate (in the sense of dimension or measurement, e.g., mg kg^{-1}) and thus usage of 'higher' or 'lower' is justified. In other cases such a rate or quantitative measure is implied. However, in response to the reviewer's concern, we have now gone through the manuscript and changed the usage of 'higher' and 'lower' in a number of places where we rates, heights or temperatures are not clearly implied and where such a change does not sound awkward.

10. There is new information presented in the conclusion, this discussion of the implications of the data for the wider context should be made in the main body of the discussion.

We have reworded the conclusions (starting on line 568) slightly but we think that we are in agreement with guidelines saying that conclusions should not be a summary, but should present results in a broader context including the significance they might have for other aspects. However, we agree that the formulation must clearly distinct between our results and what goes beyond.

11. I think it is unnecessary to present both the data in Figures 3 and 4 and the similar data in the tables, this is a bit repetitive and gives the same message. I would prefer to see the figures rather than the tables as they are much more illustrative.

We are aware that it might be a little repetitive to show the concentrations in the tables and the contents in the figures since the reader could approximate the concentrations from the graphs by dividing the contents by the mass fractions. However, our experience when presenting this study in seminars and at conferences is that this step-by-step presentation is indispensable so that the importance of effects of pasture type on P concentrations and mass fractions, which together translate into changes in P contents, can be understood. In other words: the differences between pastures types are not just an effect of changes in mass fractions, but also of P concentrations in

structural components. We also note that the result section has been intentionally structured to report concentrations (3.1) and contents (3.2) specifically to point out these different effects.
To underline the importance of both datasets we have now inserted an explanatory sentence in the introduction, lines 100 - 102: “We also note that the effect of management (or landuse) on P concentrations in different structural components is amplified when the proportion of these components on a whole soil mass basis is also affected.”

12. In figures 2 to 4, I would use a different way of representing the productive and degraded pastures rather than referring to them as either on the left or right. Use some different shading or hatching so the different environments are obvious.

Thank you for this input. Initially we had a version with different colors for degraded and productive pastures. But we became aware that for the reader it is more confusing to have different colors for the pasture types as well as the different colors for occluded macroaggregate fractions. Therefore, we would like to keep the same colors for bars of degraded and production but to indicate the pasture type below the x-axis with the letters D and P. Please see corrections in the figures.

13. I would remove figure 1, this is adequately described in the methods.

Similar to our reply to your point 11, our experience has shown us how important this scheme (Figure1) is when presenting the method to an audience or other readers who are not familiar with this procedure. Therefore, we think that it is important to keep the figure 1, even if it might be repetitive to the text, to ensure that the reader can orient himself at a glance to recognize the structural components.

Many thanks for your thorough review. Your comments helped us to improve our manuscript.

Reviewer #2: Nesper et al. investigated P fractions in soils from degraded and productive pastures in Brazil. They found that the soils from productive pastures contained more macroaggregates and that organic P was mainly present in macroaggregates. Consequently, organic P concentration was greater in productive pastures.

A main concern is how representative the soil samples used for analyses were particularly from the degraded pastures. It is not described how many samples were taken/pasture and how it was ensured that these were representative.

Yes, we agree and now include a detailed description of the sampling (lines 145-151): “For aggregate separation and P analyses, four soil sub-samples were taken from regularly spaced sampling points, at 3 m from a central point in each pasture type on all nine farms in July 2011 (Fonte et al., 2014a). Samples were carefully collected to a depth of 10 cm using a soil knife so as to incur minimum disruption of aggregates. The four sub-samples were combined, resulting in one sample per each pasture type per farm, or 18 samples in total.” Additionally, earlier measurements done in a preliminary study (Hegglin, D.J., 2011. Phosphorus status in pastures established on highly weathered tropical soils of Caqueta, Colombia, MSc thesis ETH, Zurich, 33 pp), at exactly the same sites using soil cores and taking more soil samples gave similar results (i.e., not significantly different values for concentrations of analyzed P forms).

Another problem is the high P sorption capacity of the soils. How can you be sure that P released ,by a certain extractant remained in solution? If a proportion of the released P was readsorbed, the size of the P fraction would be underestimated. This is particularly problematic in sequential fractionation because P released in earlier fractionation steps (which would be underestimated) would appear in later steps (which could be overestimated if the sorption capacity of the remaining soil was low).

Thank you for this concern, but we have not analyzed the samples using a sequential fractionation method. All analyses were run separately. In the NaOH-EDTA extracts no readsorption was possible since the soil was removed from the extracts prior to for analysis of P concentrations or addition of the enzymes to study hydrolyzable P concentrations. This is also the reason why relatively strong extractants (NaOH-EDTA and not citrate) were necessary to extract P (see reply to question 3 of reviewer 1).

The statistical analysis is strange. It seems as if pasture type and aggregate type were treated as main factors. But looking at the data and the letters, it is likely that the interaction pasture type and aggregate type was significant. Therefore there should be only one type of letters in the tables and figures for the interaction. That would also make the tables and figures easier to read and understand.

Thank you for pointing this out. We agree that the explanation in the statistical analysis section was not entirely clear and have corrected it accordingly (lines 248 – 254: "Differences between degraded and productive pastures were analysed using a linear mixed model with farms considered as blocks and treated as random variable. Likewise, differences between aggregates and fractions within pasture type were analysed using the same model since the interaction between pasture types and aggregates was not significant." We had tested the interaction between pasture type and aggregate type while preparing the paper and have not found any significance.

The finding that organic C and organic P were positively correlated is not surprising because organic P is part of the organic matter. Therefore the conclusion that organic P is mainly stored in macroaggregates is also not surprising because these aggregates also store organic C.

The question whether organic P is entirely stabilized together with organic C, or whether organic P contents (and forms) develop to some degree independently from overall soil organic matter is still a matter of debate. It is known that specific organic P compounds such as inositol hexakisphosphates are stabilized through strong complexation with metals and clay surfaces ((Celi and Barberis, 2005)) (see lines 393-395 and 414-415). The linkage between organic P and C is usually less tight than between C and N. Please see, e.g., Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C., Batten, G., (2011). Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. Geoderma 163(3-4), 197-208. These authors write in the abstract: "The weaker relationship between OP (organic P) and C for both sets of soils, compared with the relationship between N, S and C was probably also due, in part, to the wide variety of compounds in the soil OP pool which vary in their relationship with humus and the wide C:P ratio found in the soil microbial biomass."

Seen that the soils which we studied are dominated by Al- and Fe oxides and –hydroxides, one could expect a strong sorption of organic P independent from organic C. However, the close relationship that we found suggests that this was not the case.

The discussion is too long. It should be shortened focussing on the main results, not try to explain each finding, particularly not those already reported in other studies.

This comment agrees with those of reviewer 1 and we have now shortened the discussion section accordingly.

L137-141: how were the soil samples collected? I assume that the productive pastures had fairly uniform plant cover, but the degraded pastures were patchy with some patches bare and others with plants. How many soil samples did you take? How can you be sure that they are representative of the pasture? Later you give in tables and figure n=9. That indicates that only one sample per pasture was taken. Unless these samples were pooled from several samples taken in a given pasture, I doubt that they can be regarded as representative, particularly in the degraded pastures.

Please see above. The related section in Material and methods now reads:

"For aggregate separation and P analyses, four soil sub-samples were taken from regularly spaced sampling points, at 3 m from a central point in each pasture type on all nine farms in July 2011 (Fonte et al., 2014b). Samples were carefully collected to a depth of 10 cm using a soil knife so as to incur minimum disruption of aggregates. The four sub-samples were combined, resulting in one sample per each pasture type per farm, or 18 samples in total. Field moist soil clods ….."

L302-311: The finding that organic C and organic P were correlated is not surprising.

Please see the explanation above on the same matter.

Thanks again for your comments.

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Highlights

1. Tropical pasture degradation is linked to soil structural decline and reduced soil organic P.
2. Reduction of organic P affected enzyme-hydrolyzable and -stable organic P forms.
3. Microaggregates within large macroaggregates are important for organic P storage.
4. Strong correlation between organic P and C suggests similar stabilization mechanisms.

Abstract

Degradation of tropical pastures on highly weathered soils is linked to soil structural decline and associated losses of organic matter, which could both also affect soil phosphorus (P) dynamics and availability. Our aim was to elucidate these linkages by examining the effect of pasture degradation on the contents and forms of P contained within aggregate size classes and macroaggregate fractions (collectively referred to as soil structural components). We conducted a study on nine farms in a deforested part of the Colombian Amazonia, each farm with degraded and productive *Brachiaria* spp. pastures. Topsoil (0-10 cm) samples were separated by wet-sieving into aggregate size classes, and macroaggregates further separated into occluded fractions. Soils and structural components were analyzed for concentrations of total P, available P (extracted using anion exchange resins), NaOH-EDTA extractable organic and inorganic P, and enzyme-hydrolyzable organic P (simple monoester-like, DNA-like, *myo*-inositol hexakisphosphate-like) in the NaOH-EDTA extracts. Degraded pasture soils had significantly fewer large macroaggregates and more microaggregates, both with significantly lower organic P concentrations (in mg P kg^{-1} of structural component) than those in productive pasture soils. At the same time, total and extractable inorganic P concentrations in bulk soil and structural components did not differ between pasture types, suggesting a shift from organic to non-extractable P upon degradation. Soils under productive pastures contained 37% more organic P than degraded pasture soils (in mg P kg^{-1} soil), mainly in large macroaggregates. The organic P concentrations were strongly correlated with C concentrations across all soil structural components, suggesting similar stabilization mechanisms for organic P and C. In bulk soil and most structural components, around 60% of organic P was enzyme-hydrolyzable. The lower contents of all enzyme-hydrolyzable as well as enzyme-stable organic P in degraded vs. productive pasture soils indicate a reduction of all organic P classes during pasture degradation.

The large macroaggregates and, in particular, microaggregates occluded within this fraction were identified as an important site for organic P storage. Our results highlight a linkage between soil structure and organic P, both of which can play an important role in maintaining the productivity of pastures established on highly weathered soils.

Pasture degradation decreases organic P content of tropical soils due to soil structural decline

Authors:

Maike Nesper[a], Else K. Bünemann[a], Steven J. Fonte[b,d], Idupulapati M. Rao[b], Jaime E. Velásquez[c], Bertha Ramirez[c], Django Hegglin[a], Emmanuel Frossard[a], Astrid Oberson[a]

[a] ETH Zurich, Institute of Agricultural Sciences, Lindau, Switzerland
[b] Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia
[c] Universidad de la Amazonia, Florencia, Colombia
[d] Current address: Department of Plant Sciences, University of California, Davis, USA

Corresponding author: astrid.oberson@usys.ethz.ch

Abstract

Degradation of tropical pastures on highly weathered soils is linked to soil structural decline and associated losses of organic matter, which could both also affect soil phosphorus (P) dynamics and availability. Our aim was to elucidate these linkages by examining the effect of pasture degradation on the contents and forms of P contained within aggregate size classes and macroaggregate fractions (collectively referred to as soil structural components). We conducted a study on nine farms in a deforested part of the Colombian Amazonia, each farm with degraded and productive *Brachiaria* spp. pastures. Topsoil (0-10 cm) samples were separated by wet-sieving into aggregate size classes, and macroaggregates further separated into occluded fractions. Soils and structural components were analyzed for concentrations of total P, available P (extracted using anion exchange resins), NaOH-EDTA extractable organic and inorganic P, and enzyme-hydrolyzable organic P (simple monoester-like, DNA-like, *myo*-

inositol hexakisphosphate-like) in the NaOH-EDTA extracts. Degraded pasture soils had significantly fewer large macroaggregates and more microaggregates, both with significantly lower organic P concentrations (in mg P kg^{-1} of structural component) than those in productive pasture soils. At the same time, total and extractable inorganic P concentrations in bulk soil and structural components did not differ between pasture types, suggesting a shift from organic to non-extractable P upon degradation. Soils under productive pastures contained 37% more organic P than degraded pasture soils (in mg P kg^{-1} soil), mainly in large macroaggregates. The organic P concentrations were strongly correlated with C concentrations across all soil structural components, suggesting similar stabilization mechanisms for organic P and C. In bulk soil and most structural components, around 60% of organic P was enzyme-hydrolyzable. The lower contents of all enzyme-hydrolyzable as well as enzyme-stable organic P in degraded vs. productive pasture soils indicate a reduction of all organic P classes during pasture degradation. The large macroaggregates and, in particular, microaggregates occluded within this fraction were identified as an important site for organic P storage. Our results highlight a linkage between soil structure and organic P, both of which can play an important role in maintaining the productivity of pastures established on highly weathered soils.

Keywords: Amazon Basin, Colombia, tropical pastures, pasture degradation, soil aggregation, phosphorus, phosphorus forms, enzyme additions

1. Introduction

Highly weathered soils of the humid tropics typically have low total and available phosphorus (P) contents and P is often the primary limiting nutrient to plant growth (Nziguheba and Bünemann, 2005; Vitousek et al., 2010). On deforested areas in South

America, pastures sown with introduced grasses (*Brachiaria* spp.) represent a dominant land use, and the majority of pastures exist in some stage of degradation (Jimenez and Lal, 2006). Pasture degradation is understood as a marked reduction in livestock production due to a significant decrease in plant biomass production and invasion of non-palatable plant species, and it leads to appearance of bare soil patches, soil compaction and reduced soil microbial biomass (Boddey et al., 2004; de Oliveira et al., 2004). Pasture degradation has enormous economic implications. For example, in Brazil every year about 8 million hectares of degraded pastures require considerable investment for renewal and/or recovery (Jank et al., 2014), with estimated costs of 100 to 200 US$ ha^{-1}, i.e., around 1 billion US$ in total (FAO, 2006). Soils of degraded pastures have been shown to store less carbon (C) than soils of productive pastures (Asner et al., 2004; Fonte et al., 2014). Pasture over-grazing and reduced pools of available nitrogen (N) and P are seen as the principal causes of degradation (Dias-Filho et al., 2001; Fisher et al., 2007). While the importance of N cycling and availability has been demonstrated previously (Boddey et al., 2004), a mechanistic understanding of the role of P in maintaining pasture productivity is missing.

Because most inorganic P is strongly sorbed, organic P has been suggested to play a critical role in sustaining P availability in highly weathered soils (Tiessen et al., 1984; Turner et al., 2006). Biological P cycling through plant uptake, residue decomposition and microbial turnover, which includes synthesis of organic P, appears to be crucial to protect P from sorption and to maintain P availability in highly weathered tropical soils (Oberson et al., 2006). Organic P constitutes 16% to 65% of total P in highly weathered soils (Nziguheba and Bünemann, 2005).

A single-step procedure involving sodium hydroxide and EDTA (ethylenediaminetetraacetate) is often used for the extraction of soil organic P from strongly weathered tropical soil (Turner, 2008). Additions of enzymes to various soil extracts have been used to characterize hydrolyzable forms of organic P, as reviewed by Bünemann (2008), e.g., addition of acid phosphatase or phytase to soil water extracts (Fox and Comerford, 1992; Shand and Smith, 1997). As different enzymes hydrolyze different organic P bonds, this approach delivers information on the chemical form of organic P in soils (He and Honeycutt, 2001; Turner et al., 2002a). Based on P release by acid phosphatase, phytase and nuclease from defined organic P compounds, Annaheim et al. (2013) classified the hydrolyzed P into simple monoesters, *myo*-inositol hexakisphosphate and nucleic acids. Keller et al. (2012) used the same approach to classify organic P in NaOH-EDTH extracts. Enzyme-hydrolyzable organic P identified by phytase addition to EDTA extracts was shown to be greater in Oxisols under no till than with conventional tillage (Pavinato et al., 2010), suggesting that enzyme-hydrolyzable organic P is a sensitive indicator for the impacts of soil management on soil P status.

Soil aggregation is considered important for regulating the storage and turnover of soil organic C (Six et al. 2000) and nitrogen (N) (Bosshard et al., 2008). Relatively few studies have examined the effect of aggregation on P and these have suggested that P availability, P concentrations and forms can be influenced by aggregation (McDowell et al., 2007; Wang et al., 2001). For example, P uptake was greater in plants grown in large aggregates (2-6 mm) than in plants grown in small aggregates (< 0.5 mm) of highly weathered Oxisols because P bound to large aggregates was more readily desorbed (Wang et al., 2001). The water extractable P concentration decreased with smaller aggregate sizes in cropped soils in Canada (Messiga et al., 2011). The concentrations of orthophosphate, monoesters, diesters and pyrophosphates determined by ^{31}P-nuclear magnetic resonance (^{31}P-NMR) spectroscopy increased with decreasing aggregate size in a soil from New Zealand, while phosphonates and polyphosphates were unaffected (McDowell et al., 2007). We also note that the effect of management (or landuse) on P concentrations in different structural components is amplified when the proportion of these components on a whole soil mass basis is also affected.

In a recent study, the distribution of soil mass among aggregate size classes was found to differ between degraded and productive pasture soils (Fonte et al., 2014). Although soils of both pasture types had a high aggregate stability, the proportion of large macroaggregates (>2000 µm) was significantly higher in productive (65 g 100 g^{-1}) than in degraded pasture soils (56 g 100 g^{-1}). Soil of productive pastures had 20% higher total C and N contents (in g kg^{-1} soil) than degraded pastures. These differences in total soil organic matter (SOM) between pasture types were largely explained by a greater C content in the large macroaggregate fraction, and more specifically in the microaggregates (53-250 µm) occluded within this macroaggregate fraction (Mmicros). Interestingly, there was no difference in total P content between pasture types, but organic P content was found to be nearly 40% greater in soils of productive vs. degraded pastures. The findings of Fonte et al. (2014) suggest that different organic P contents in the bulk soils of productive and degraded pastures could be related to differences in soil structure and the distribution of C across aggregate fractions. Specifically, these results lead us to hypothesize that greater organic P content is associated with the greater protection of SOM in the Mmicros fraction of productive pastures.

To further elucidate the role of aggregation in the P status of highly weathered tropical soils we examined the distribution of organic, inorganic and available P across aggregates and occluded macroaggregate fractions of the same degraded and productive pasture soils studied by Fonte et al. (2014). To obtain information on organic P forms, we determined enzyme-hydrolyzable organic P classes by enzyme additions. Finally, we studied the relationship between C and organic P across the different soil structural components.

2. Material and Methods

2.1 Site description, experimental design and soil sampling

The study was conducted on nine farms located in the deforested Amazon region of Colombia. All farms are situated within a 30 km radius of the city of Florencia, in the Department of Caquetá (1°36′50″N 75°36′46″W) with an average elevation of 280 m.a.s.l. The region has a humid tropical climate with a mean annual precipitation of 3400 mm and a mean annual temperature of 25°C. The mildly undulating topography is characterized by acid soils, mainly Oxisols and Ultisols (Mosquera et al., 2012) with textures in the 0-10 cm soil layer ranging from silty clay to sandy clay loam (Fonte et al., 2014).

The design of the study has been described in Fonte et al. (2014). Briefly, on each of the nine farms, areas of productive and degraded pasture were identified together with farmers. Degraded pastures had clear signs of degradation (e.g., patches of bare soil, invasion of herbaceous weeds and grasses other than the sown species, significant or total loss of previously sown *Brachiaria* spp.), while productive pastures were those dominated by *Brachiaria* spp. (mainly *B. humidicola* CIAT 679 and *B. decumbens* CIAT 606). Productive pastures had significantly greater live and litter biomass than degraded pastures (Table 1). Degraded and productive pastures had similar topography and age since pasture establishment. All farms experienced similar management histories, with forest cleared at least 40 years earlier. Basic soil properties are shown in *Table 1*.

For aggregate separation and P analyses, four soil sub-samples were taken from regularly spaced sampling points, at 3 m from a central point in each pasture type on all nine farms in July 2011 (Fonte et al., 2014). Samples were carefully collected to a depth of 10 cm using a soil knife so as to incur minimum disruption of aggregates. The four sub-samples were combined, resulting in one sample per each pasture type per farm, or 18 samples in total. Field moist soil clods were gently broken along natural planes of weakness, so that they also passed the 8 mm sieve. Earthworms, termites, plant debris and stones were removed. The soil was dried at 50°C and shipped to the Soil Physics laboratory of the International Center for Tropical Agriculture (CIAT) in Cali, Colombia for aggregate separation.

2.2 Soil and macroaggregate fractionation

We analyzed sub-samples of the various aggregate size classes and occluded macroaggregate fractions isolated by Fonte et al. (2014). We here summarize the fractionation method. Soil samples were first wet-sieved based on Elliott et al. (1986) into large macroaggregates (> 2000 µm), small macroaggregates (250-2000 µm), microaggregates (53-250 µm) and the silt&clay (< 53 µm) fraction (Fig. 1). To this end, 80 g of soil were submerged in deionized water on a 2000 µm sieve and slaked for 5 minutes. The soil was then sieved by oscillating the sieve in and out of the water 50 times over a period of 2 minutes. Macroaggregates remaining on the sieve were then washed into a pre-weighed aluminium pan for drying. Soil which passed through the sieve was transferred to a smaller sieve and the sieving process repeated with a 250 µm and with a 53 µm sieve. All free aggregate size classes were dried in the oven at 50°C and their mass determined. The silt&clay fraction passing through the 53 µm sieve was associated with a large volume of water (approx. 3 l) and the slurry was dried together in a large pan. Before further processing, stones and organic material > 2 mm, which are not typically incorporated into soil aggregates, were removed and weighed.

Macroaggregates were further fractionated after the method of Six et al. (2000a) to obtain occluded fractions within both large and small macroaggregates (Fig. 1). Briefly, 6 g of macroaggregates were submerged in water for 24 h and kept at 4°C for slaking. Macroaggregates were then placed on a modified 250 µm sieve, submerged in water and shaken horizontally (250 rpm) with 40 stainless steel beads (6 mm diameter) until all macroaggregates were broken up (3-8 min). While macroaggregates were being ruptured, particles smaller than 250 µm were transported by a constant water flow to a 53 µm sieve below. These particles were then wet-sieved as described above to separate microaggregates within macroaggregates (Mmicros) from silt&clay occluded in macroaggregates (Msilt&clay). The fraction remaining on the 250 µm sieve constituted the coarse particulate organic matter (cPOM) and sand fraction. As for the free aggregate classes, all macroaggregate occluded fractions were dried in the oven at 50°C. A subsample was taken from bulk soil, aggregates and occluded fractions and ground to a powder using a ball mill (Retsch, GmbH, Germany) prior to analysis.

2.3 Nutrient analyses and enzyme addition assay

Concentrations of total P in bulk soil, aggregates and occluded fractions were measured colorimetrically (Ohno and Zibilske, 1991) after heat digestion of 200 mg sample with H_2O_2, H_2SO_4, Se, and Li_2O_4S at 340°C for 2 h 30 min (Anderson and Ingram, 1993). Plant available P was extracted from 1 g of sample using anion-exchange resin strips saturated with HCO_3^- after Kouno et al. (1995). Organic P was extracted from 500 mg of sample with 5 ml NaOH-EDTA solution (Bowman and Moir, 1993). Inorganic P in the NaOH-EDTA extracts was measured colorimetrically (Ohno and Zibilske, 1991). Another subsample of the extract was digested to obtain NaOH-EDTA total P by autoclaving the extract with H_2SO_4 and ammonium persulphate, subsequent neutralization and colorimetric measurement. Organic P was calculated as the difference between total and inorganic P in the NaOH-EDTA extract. The difference between total soil P (obtained with the H_2O_2-H_2SO_4 digestion described above) and NaOH-EDTA total P is non-extractable P.

The following enzymes were added to the NaOH-EDTA extracts in a microplate assay (Keller et al., 2012): (1) Acid phosphatase (EC 3.1.3.2) from potato, Sigma Chemicals, Product No. P1146, (2) Nuclease P1, from *Penicillium citrinum*, Sigma Chemicals, Product

No. N8630 together with acid phosphatase, and (3) Phytase from *Peniophora lycii* (RONOZYME® NP (M), DSM Nutritional Products Denmark). After adding the enzymes and MES buffer (adjusted to a pH of 5.2), the microplate was incubated at 37°C for 48 h, followed by colorimetric determination of molybdate reactive P concentrations (Ohno and Zibilske, 1991) in enzyme-amended and non-amended extracts. Mean concentrations were calculated from four analytical replicates. The concentration of P released by a given enzyme was calculated as the difference in P measured in soil extracts incubated with and without the enzyme preparation, additionally accounting for the background concentration of molybdate reactive P in the enzyme preparation. This background was significant only for the phytase preparation. The differences were also corrected for P spike recoveries measured in each treatment, which were usually greater than 95%. Enzyme-hydrolyzable organic P was classified based on the enzyme specificity determinations of Annaheim et al. (2013):

(1) Simple monoester-like organic P: P in simple monoesters like glycerol phosphate, glucose-6-phosphate, in anhydrides and in condensed organic compounds like pyrophosphate and ATP; *P released by acid phosphatase.*

(2) DNA-like organic P: P in nucleic acids; *(P released by nuclease plus acid phosphatase) – (P released by acid phosphatase).*

(3) InosP-like organic P: P in *myo*-inositol hexakisphosphate; *(P released by phytase) – (P released by acid phosphatase).*

The sum of all three enzyme-hydrolyzable P classes is the enzyme-hydrolyzable organic P. Enzyme-stable organic P in NaOH-EDTA extracts was calculated as the difference between organic P and enzyme-hydrolyzable organic P.

The P contents (mg P kg^{-1} soil) in the soil structural components were obtained by multiplying the P concentration of each component (mg kg^{-1}) by its mass (kg component kg^{-1} soil). The analyses were conducted on the nine replicate samples of bulk soil and structural components per pasture type. Exceptions due to limited sample mass are given in footnotes of tables and figures. The determinations of total, organic and available P were repeated at least three times for each sample, and the average of the three analytical replicates used. The sum of P extracted from the aggregate size classes (large and small macroaggregates, free microaggregates, free silt&clay) had the following average (± standard deviations) percentages of P extracted from the bulk soil: 101±12% for total P; 104±23% for available P; 100±21% for organic P; and 108±24% for enzyme-hydrolyzable organic P. The sum of P forms extracted from the fractions occluded in large and small macroaggregates, in percentage of total P of intact large and small macroaggregates, was on average 93±10% for total P, 105±23% for organic P, and 97±23% for enzyme-hydrolyzable organic P and 55±15% for available P. The recovery of available P does not include cPOM, as it could not be analyzed due to insufficient sample.

2.4 Statistical analysis

The statistical analyses were completed with R 2.12.2 (© 2011 The R Foundation for Statistical Computing). Comparisons and other calculations were carried out using mean values of the analytical replicates. All data was tested for normal distribution by the Shapiro normality test and transformed using natural log or arcsin when necessary. Differences between degraded and productive pastures were analysed using a linear mixed model with farms considered as blocks and treated as random variable. Likewise, differences between aggregates and fractions within pasture type were analysed using the same model since the interaction between pasture types and aggregates was not significant. Soil texture (clay + silt) was included as a covariate in the linear mixed model since texture has been shown to have a significant influence on soil aggregation (Fonte et al., 2009), P concentration (McGrath et al.,

2001) and SOM content (Feller and Beare, 1997). Significance level for all statistical tests was set at $p = 0.05$, unless reported otherwise.

3. Results

3.1 Phosphorus concentrations in bulk soil, aggregates and fractions

Concentrations of total and available P in the bulk soil highlight the low P status of the soils, with average total P concentrations of 362 mg P kg^{-1} and less than 7 mg kg^{-1} of available P extracted using anion exchange resins (Table 2). Organic P concentration was significantly (37%) higher in the bulk soil of productive vs. degraded pasture soils, while total and available P concentrations were similar. Organic P constituted on average 19% of total P in degraded and 26% in productive pasture soils. On average 58% of the organic P was enzyme-hydrolyzable and simple-monoester-like P was the greatest identified organic P class in the bulk soil (Fig. 2). While there were hardly any significant differences between degraded and productive pasture soils in identified organic P classes, degraded pasture soils had significantly lower enzyme-stable organic P concentrations (Table 3).

Inorganic P and available P concentrations decreased with decreasing aggregate size classes while total and non-extractable P concentrations did not significantly vary across aggregate size classes (Table 2). Organic P was lowest in free microaggregates and highest in silt&clay. The total of enzyme-hydrolyzable organic P did not significantly change with aggregate size class (Table 2), but the concentrations of InosP-like P were highest in the small macroaggregates, and in productive pasture soils also the enzyme-stable organic P concentrations were significantly affected by aggregation (Table 3). Total, inorganic and available P concentrations did not significantly differ between aggregate size classes of productive vs. degraded pasture soils (Table 2). In contrast, the organic P concentrations were significantly higher in the large and small macroaggregates and in the free microaggregates of the productive than in those of the degraded pasture soils. Macroaggregates from productive pasture soils also had significantly greater enzyme-stable organic P concentrations and InosP-like P was higher in free microaggregates from productive pastures while concentrations of other identified organic P forms were little affected by pasture type (Table 3).

Within macroaggregates, available P concentrations were higher in the occluded microaggregates than in the silt&clay (Table 4). Inorganic P, organic P and enzyme-hydrolyzable organic P concentrations were lowest in the cPOM. Accordingly, concentrations of identified organic P forms were lowest in cPOM (supplementary Fig. 1). At the same time, cPOM was characterized by the highest proportion of enzyme-hydrolyzable P (Fig. 2). Similar to the findings for macroaggregates, the pasture type also affected the P concentrations of the occluded fractions (Table 4). The available P concentrations were significantly lower in the Mmicros and in the Msilt&clay fractions of small macroaggregates of the degraded than the productive pastures. Likewise, organic P concentrations were lower in the Mmicros of large macroaggregates of degraded pastures. These differences were largely reflected in the enzyme-hydrolyzable organic P. All identified P forms, except for enzyme-stable organic P, showed the same characteristic of lower P concentrations in the Mmicros of degraded than of productive pastures.

3.2 Phosphorus content in aggregates

Soil structure significantly differed between productive and degraded pasture soils (Fig. 3.1), as shown by Fonte et al. (2014). Differences in aggregate distribution and P concentrations resulted in significantly different contents of P forms, on a mg per kg^{-1} soil basis, between productive and degraded pastures (Fig. 3.2-3.6). Inorganic, organic and enzyme-hydrolyzable organic P contents were significantly greater in large macroaggregates of productive than degraded pastures. In contrast, the total, inorganic and available P contents were significantly greater in free microaggregates and silt&clay of degraded than productive pastures (inorganic P only in the microaggregates), but these are small pools due to their low overall proportion of the total soil mass (Fig. 3.1).

Productive pasture soils contained significantly more enzyme-stable organic P and tended to have more InosP-like P than degraded pasture soils (Fig. 4). These differences were for several organic P forms significant in the large macroaggregates and the Mmicros and Msilt&clay fractions, and were consistent with the overall greater organic P and enzyme-hydrolyzable organic P in these fractions (Fig. 3, 4).

Contents of all the P forms in aggregates and fractions decreased in an order similar to the one of the distribution of their mass fraction (Fig. 3.1). Thus, the Mmicros of large and small macroaggregates had highest contents of all P pools while cPOM, free microaggregates and free silt&clay presented small pools (Fig. 3.2-3.6).

3.3 Relationship between carbon and organic P

Organic P and C concentrations were highly correlated across all structural soil components (Fig. 5, with C concentrations from Fonte et al. (2014)). As correlation coefficients and regression functions were similar for degraded and productive pasture soils, Fig. 5 includes data from both pasture types. It shows that concentrations of C and organic P changed in parallel across aggregate size and fraction. We note that the C and P concentrations for one cPOM sample were several times higher than that observed in all other cPOM samples because of its lower sand content. The C to organic P ratio varied between 240 (silt&clay) and 890 (cPOM) (supplementary Table 1). It tended to decrease with aggregate size and was lowest in the silt&clay fraction. The C to organic P ratios were not significantly different between productive and degraded pasture soils.

4. Discussion

4.1 Aggregation affects organic and inorganic P contents

Our results suggest that soil aggregation is crucial in maintaining organic P contents in tropical grassland soils. In particular, most organic P was stored within large macroaggregates, and more specifically within microaggregates occluded within macroaggregates (Mmicros, Fig. 3.4). Since the concentrations of enzyme-hydrolyzable organic P largely changed in parallel with organic P across aggregates and occluded fractions, the Mmicros fraction was also an important site for the storage of enzyme-hydrolyzable P forms and of enzyme-stable P (Fig. 4). McDowell et al. (2007) also found greater concentrations of organic P forms in aggregate size classes similar to our macroaggregates, than in unsieved soils or aggregates < 250 µm of a cultivated soil from New Zealand. However, these macroaggregates were not fractionated further for P analysis. The distribution of organic P among aggregates and fractions was similar to the distribution previously observed for C (Fonte et al., 2014). Also, Denef et al. (2007) identified the Mmicros to be a preferential site of C accumulation in Oxisols. Likewise, we found this same fraction to be important for organic P storage. The organic P concentration was significantly higher in both Mmicros of large and small macroaggregates than in the free microaggregates, for degraded and productive pastures.

Aggregate formation and related physical protection of SOM within aggregates are largely driven by biotic processes (Six et al., 2000a; Tisdall and Oades, 1982). However, in highly weathered tropical soils dominated by 1:1 clays and Fe and Al (hydr)oxides, sorption of SOM to mineral particles provides an additional, important stabilization mechanism (Dungait et al., 2012; Six et al., 2000b). These particles can also strongly sorb organic P forms (Ognalaga et al., 1994; Pavinato et al., 2010). The strong correlation between C and organic P suggests that soil aggregation affects the stabilization of C and organic P to a similar degree (Fig. 5). Likewise, the decrease of the C to organic P ratios with aggregate size (supplementary Table 1) was in line with the decrease in the C to N ratios in the same aggregates (Fonte et al., 2014). Decreasing C to N ratios have been interpreted as more processed forms of SOM in aggregates of smaller size (Six et al., 2001).

Aggregation also significantly affected the concentration and contents of inorganic P, with inorganic P concentrations significantly decreasing with aggregate size (Table 2). Similarly, macroaggregates had greater concentrations of water extractable and/or EDTA extractable molybdate reactive P than microaggregates and the silt&clay sized particles in cropped Ultisols (Green et al., 2006). Due to highest concentration and proportion of total soil mass, the macroaggregates in our study contained a substantial amount of the NaOH-EDTA extractable inorganic P (Fig. 3.3). As for organic P, the occluded microaggregates were an important storage site and had higher inorganic P concentrations than the free microaggregates ($p < 0.001$). Likewise, they were a principal storage site of available P (Fig. 3.5). The importance of aggregation was also indicated by the higher available P concentrations in the occluded than in the free microaggregates or the silt&clay fraction. This suggests that upon disaggregation, reactive sorption sites increase P sorption, which is in line with the stronger sorption on silt&clay reported earlier by Wang et al. (2001). Likewise, sorption was strongly increased following the destruction of organic matter by a H_2O_2 treatment of highly weathered soils dominated by 1:1 clays, as this organic matter had probably masked sorptive surfaces through organo-mineral aggregation (Frossard et al., 1992). Apparently, the increase in P sorption upon disaggregation also occured during the analyses of our study, as the sum of available P extracted from fractions occluded in large and small macroaggregates was only 55±14% of the available P extracted from the intact macroaggregates. Still, this sum does not include the cPOM, which could not be analyzed for available P due to insufficient sample.

4.2 Aggregation affects organic P forms

In the bulk soil and all structural components except the cPOM, about 60% of the extracted organic P was hydrolyzed by the added enzymes. This was a higher proportion than the 33-39% found in cropped temperate zone soils (Keller et al., 2012), but similar to the average of 58% of enzyme-hydrolyzable organic P in NaOH-EDTA extracts from soils of different climatic zones and land use (Jarosch et al., 2014). The high proportion of enzyme-hydrolyzable organic P supports the overall good physical protection of SOM and organic P in the studied soils, as described above and in Fonte et al. (2014).

Because inositol hexakisphosphates are stabilized through strong complexation with metals and clay surfaces (Celi and Barberis, 2005), they are often the dominant class of organic P in soils (Turner et al., 2002b) and we expected to see that confirmed in our study. InosP-like P was also the most abundant identified organic P class in enzyme addition studies on cropped and permanent grassland soils from the temperate zone (He et al., 2004; Keller et al., 2012). However, in our study the organic P contained in the soils and their structural components was not dominated by InosP (7-37% of organic P, Fig. 2). Our results corroborate Turner et al. (2007) who found, along a 120,000-year postglacial chronosequence in New Zealand, that proportions of organic P classes change with soil age, such that *myo*-inositol hexakisphosphate declines with increasing soil age and that inositol phosphates and other monoesters have similar concentrations in highly weathered soils. In another study using ^{31}P-NMR spectroscopy on NaOH-EDTA extracts, even no inositol phosphates were detected in an Oxisol under forest (Vincent et al., 2010).

We also expected that the concentrations as well as the proportions of organic P classes would differ between structural components due to aggregation-related changes in physico-chemical stabilization. Indeed, the concentrations and proportions of DNA-like P increased with decreasing aggregate size, which agrees with findings of McDowell et al. (2007) for diesters. This could be due to adsorption of DNA onto clays (Greaves and Wilson, 1969) or to microorganisms associated with the clay particles (van Gestel et al., 1996). On the other hand, apart from the greater concentration of InosP in the small macroaggregates, Inos-P and simple monoester-like P changed little with aggregation. This might be because also simple monoesters like glucose-1-phosphate get readily sorbed on Fe oxides such as goethite (Ognalaga et al., 1994). Still, the relatively high proportions of simple monoester like P might be somewhat overestimated due to the degradation of some orthophosphate diesters in studies involving alkaline extraction (Turner et al., 2003b). RNA and some phospholipids (phosphatidyl choline) were rapidly degraded during alkaline extraction and analysis (Makarov et al., 2002; Turner et al., 2003a). In contrast, DNA, other phospholipids, and orthophosphate monoesters were more stable (Turner et al., 2003a).

Our aim was to characterize total organic P using NaOH-EDTA extraction in combination with enzyme additions. The observed small differences in enzyme-hydrolyzable P between structural components do not preclude that differences in enzyme-hydrolyzable P would be seen in milder extracts, such as citrate (Hayes et al., 2000), water or bicarbonate (George et al., 2007). Such milder extractants can only be used in soils in which they extract sufficient P to observe an increase in molybdate-reactive P following enzyme addition.

About 40% of the extracted organic P was not hydrolyzed by the added enzymes and its form therefore remains unidentified. Keller et al. (2012) suggested that this organic P might correspond to the broad signal found in the monoester region of ^{31}P-NMR spectra (Doolette and Smernik, 2011), which might be due to P associated with large humic compounds (Doolette et al., 2011). Because of incorporation into humic substances, it may not be accessible to enzymatic hydrolysis as shown for organic P incorporated into model humic polymers (Brannon and Sommers, 1985).

Contrary to our expectations, the proportion of enzyme-hydrolyzable organic P was similar in all structural components except the cPOM which had a significantly higher proportion of enzyme-hydrolyzable organic P forms. The cPOM consists mainly of relatively undecomposed plant debris (von Lützow et al., 2007) and P from fungi decomposing these plant residues (Salas et al., 2003), which explains that organic P contained therein will more readily undergo enzymatic hydrolysis. The more labile character of cPOM was furthermore indicated by the faster turnover time of C contained in cPOM than of C contained in any other structural component, as derived from the $\delta^{13}C$ signatures (Fonte et al., 2014). In contrast, the lower and similar proportions of enzyme-hydrolyzable P in all other structural components were in line with more similar C turnover times among these fractions.

Nevertheless, the pasture type affected the concentrations and contents of enzyme-hydrolyzable and enzyme-stable organic P forms in soils and several structural components. This indicates that all organic P forms underwent transformations during pasture degradation.

4.3 Impact of pasture degradation on P forms and content is related to soil structure decline

Comparison of the degraded and productive pasture soils illustrates the linkage between soil structure, P status and the implications for P dynamcis. Fonte et al. (2014) found greater contents of organic P in the bulk soils of productive vs. degraded pastures, despite a lack of differences in total P content, and suggested that this is related to differences in soil structure. More specifically, their findings suggested that greater organic P content is associated with the greater protection of SOM in the Mmicros fraction of productive pastures. The P analyses presented here support this hypothesis.

Soils of productive pastures contained more large macroaggregates than degraded pasture soils (Fig. 3.1) (Fonte et al., 2014). Together with higher organic P concentrations in this fraction for productive vs. degraded pastures, this translated into greater organic P storage in the large macroaggregates of productive pastures. The organic P concentrations were higher in several other structural components of productive vs. degraded pasture soils as well, and the C and organic P concentrations were strongly linked across all structural components (Fig. 5). The loss of C (Asner et al., 2004; Fonte et al., 2014) and organic P from degraded pasture soils appear to be linked, as the C to organic P ratios were similar for degraded and productive pasture soils across all structural components and the bulk soil (supplementary Table 1). Concomitant loss of C and organic P was earlier reported for cultivated soils in semi-arid northeastern Brazil (Tiessen et al., 1992) and in North American prairie soils (Tiessen et al., 1982).

Several mechanisms could contribute to lower organic P contents in degraded than productive pasture soils, which are i) reduced organic inputs, ii) less organic P synthesis due to lower microbial activity, iii) loss of organic P due to reduced physical protection of SOM in aggregates and iv) due to prolonged anoxic soil conditions. These mechanisms are interlinked, and are discussed in detail below.

The input of C and organic P contained in aboveground plant litter (Table 1) and in belowground residues, because of lower root density (Fonte et al., 2014), was lower in degraded than in productive pastures. Furthermore, due to the lower amount of forage offered by degraded pastures, less C and organic P in animal manure were recycled back to the soil.

Carbon input stimulates microbial growth and activity (Bünemann et al., 2004a). Microbial growth is related to microbial P uptake, which involved moderately available soil P in highly weathered tropical soils (Bünemann et al., 2004b), and which is related to organic P synthesis (Bünemann et al., 2008). Soil organic P has been suggested to be largely of microbial origin (Magid et al., 1996). Thus, the lower microbial activity in degraded vs. productive pasture soils as suggested by lower microbial respiration (Table 1), could result in lower organic P synthesis.

Aggregate formation is largely driven by biotic processes (Six et al., 2000a; Tisdall and Oades, 1982), such as the activity of plant roots, microorganisms and earthworms (Fonte et al., 2012; Guggenberger et al., 1999). Since organic inputs drive soil microbial and faunal activity (Bünemann et al., 2004a; Lavelle et al., 2001), lower inputs in degraded pastures likely result in lower soil aggregation and, in consequence, lower physical protection of SOM, including organic P. The lower organic P contents at similar total P contents indicate that due to lower protection, this P was mineralized. Because several soil structural components of degraded pastures contained less extractable inorganic and/or available P than in productive pasture soils, at similar total P concentrations, the mineralized organic P was most probably converted into non-extractable P forms (Tables 2, 4). Conversion of mineralized organic P into non-extractable P has been reported in earlier studies on tropical soils, e.g., from Brazil, where SOM degradation following the cultivation of a forest soil transformed organic P into non-extractable forms (Tiessen et al., 1992). This process was irreversible, despite fallow periods and a return of SOM to former levels (Tiessen et al., 1992). We have not studied the reversibility of organic P decline in our study, but we observed a similar organic P decline as Tiessen et al. (1992) (37% in our study vs. 30%). Thus, inappropriate tropical pasture management appears to have resulted in similar organic P losses to those observed with cultivation.

Degraded pastures soils are often compacted (Boddey et al., 2004), as illustrated by the greater values of bulk density of degraded vs. productive pasture soils studied here (Table 1). Compacted soils may undergo longer phases of waterlogging than non-compacted soils. Similar to observations for inorganic P (Schärer et al., 2009), prolonged anoxic conditions associated with compaction could increase the desorption of organic compounds (Peretyazhko and Sposito, 2005), making them more suceptible to enzymatic attack and also potentially more prone to losses. Concentrations of dissolved inorganic and organic P were sometimes greater in river waters from pasture-dominated as compared to forested watersheds in the Brazilian Amazon (Neill et al., 2001), suggesting that pastures lose more P than forests. However, Neill et al. (2001) did not investigate the status of the pastures, and the significance of specific nutrient loss pathways such as surface runoff, erosion and leaching remains unknown. Considering the similar total P contents of degraded and productive pastures soils, we have no indication that there was a net loss of P from degraded pastures in our study. This also suggests that there was no net P loss via grazing animals which could be explained by mineral P supplementation to grazing cattle (Dias-Filho et al., 2001).

The decline of organic P associated with degradation affected all organic P forms. This suggests that physical protection in aggregates is an important mechanism of organic P stabilization, as for overall SOM (Denef et al., 2007; Six et al., 2000a).

5. Conclusions

This study shows a clear linkage between soil structure and organic P. Large macroaggregates, and in particular the microaggregates occluded within macroaggregates, were identified as an important site of organic P storage. Soils under degraded pastures contained fewer large macroaggregates in which C and organic P can be physically protected. The observed reduction of organic P, in turn, affected all identified enzyme-hydrolyzable P forms as well as enzyme-stable P.

Degraded and productive pasture soils and their structural components had similar total P concentrations. Thus, we have no indication that soil P depletion was causing pasture degradation. However, the supply of plant available P could be lower in degraded than productive pasture soils because of reduced biological P cycling and because of increased P sorption due to reduced aggregation.

About 60% of the extracted organic P was hydrolyzed by the added enzymes. Contrary to our expectations, this proportion was similar in all soil structural components, except in the cPOM. This is the first time that enzymatic organic P characterization has been applied to NaOH-EDTA extracts obtained from aggregate size classes and their occluded fractions. Since highly weathered tropical soils are specific in their mineralogy, similar studies on temperate zone soils are needed to understand the effect of soil structure on organic forms and their hydrolyzability.

Our findings suggest that sustaining P availability in highly weathered tropical soils requires the maintenance of intact soil structure. Sustainable pasture management needs grazing management that prevents physical damage to soils and sustains organic matter return to stimulate overall soil biological activity and nutrient cycling. Restoration of degraded pasture soils requires reformation of soil structure through enhanced biological activity and may need improved forage or crop components that stimulate specific P mobilization mechanisms to bring recalcitrant P back into the cycle.

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Figure legends

Fig. 1. Bulk soil separation into large macroaggregates, small macroaggregates, microaggregates and silt&clay after Elliott (1986) and subsequent fractionation of macroaggregates into coarse particulate organic matter and sand (cPOM), microaggregates within macroaggregates (Mmicros) and occluded silt&clay (Msilt&clay) after Six et al. (2000a).

Fig. 2. Proportion of enzyme-hydrolyzable P forms and enzyme-stable P in organic P of the bulk soil, the aggregate size classes and the occluded macroaggregate fractions. Macros = macroaggregates, D = degraded pastures, P = productive pastures. For acronyms refer to the caption of Fig.1. Means and standard errors from n = 9, except for i) silt&clay (n = 6 (degraded) and n = 4 (productive)), ii) cPOM (n = 3) and Msilt&clay of large macroaggregates (n = 7 (degraded)), iii) cPOM (n = 4) and Msilt&clay of small macroaggregates (n = 7 (degraded) and n = 8 (productive)).

Fig. 3. 1) Mass distribution of aggregates and occluded fractions, contents of 2) total P, 3) inorganic P, 4) organic P, 5) available P and of 6) enzyme-hydrolyzable organic P, in aggregate size classes and occluded fractions, in mg kg^{-1}soil. Black outlines are contents in aggregate size classes and filled bars are sums of occluded macroaggregate fractions. For acronyms see caption of Fig.1, D = degraded pastures, P = productive pastures. Means of n = 9. Different letters between two bars denote significant differences ($p < 0.05$) or a trend ($p < 0.1$) between pasture types, with letters outside the bars being assigned to aggregate size

classes, and letters inside bars being assigned to occluded macroaggregate fractions. Error bars denote standard error of content in aggregate size classes.

Fig. 4. Contents of simple monoester-, DNA- and InosP-like organic P in aggregate size classes and occluded macroaggregate fractions, in mg P kg^{-1} soil. For acronyms see caption of Fig.1, D = degraded pastures, P = productive pastures. Means from n = 9 except for cPOM (n = 3) and silt&clay (n = 7 (degraded)) of large macroaggregates, cPOM (n = 3) and silt&clay (n = 7 (degraded) and n = 8 (productive)) of small macroaggregates. Different letters between two bars denote significant differences ($p < 0.05$) or a trend ($p < 0.1$) between pasture types, with letters outside the bars being assigned to aggregate size classes, and letters inside bars being assigned to occluded macroaggregate fractions. Black outlines are contents in aggregate size classes and filled bars are contents of summed occluded macroaggregate fractions. Error bars denote standard error of content in aggregate size classes.

Supplementary Fig. 1. Concentrations of specific P forms in the occluded macroaggregate fractions, in mg kg^{-1} fraction. Different lower case letters denote significant differences ($p < 0.05$) between pasture types. Different capital letters indicate significant differences between the fractions.

Pasture degradation decreases organic P content of tropical soils due to soil structural decline

Authors:

Maike Nesper[a], Else K. Bünemann[a], Steven J. Fonte[b,d], Idupulapati M. Rao[b], Jaime E. Velásquez[c], Bertha Ramirez[c], Django Hegglin[a], Emmanuel Frossard[a], Astrid Oberson[a]

[a] ETH Zurich, Institute of Agricultural Sciences, Lindau, Switzerland
[b] Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia
[c] Universidad de la Amazonia, Florencia, Colombia
[d] Current address: Department of Plant Sciences, University of California, Davis, USA

Corresponding author: astrid.oberson@usys.ethz.ch

Abstract

Degradation of tropical pastures on highly weathered soils is linked to soil structural decline and associated losses of organic matter, which could both also affect soil phosphorus (P) dynamics and availability. Our aim was to elucidate these linkages by examining the effect of pasture degradation on the contents and forms of P contained within aggregate size classes and macroaggregate fractions (collectively referred to as soil structural components). We conducted a study on nine farms in a deforested part of the Colombian Amazonia, each farm with degraded and productive *Brachiaria* spp. pastures. Topsoil (0-10 cm) samples were separated by wet-sieving into aggregate size classes, and macroaggregates further separated into occluded fractions. Soils and structural components were analyzed for concentrations of total P, available P (extracted using anion exchange resins), NaOH-EDTA extractable organic and inorganic P, and enzyme-hydrolyzable organic P (simple monoester-like, DNA-like, *myo*-

inositol hexakisphosphate-like) in the NaOH-EDTA extracts. Degraded pasture soils had significantly fewer large macroaggregates and more microaggregates, both with significantly lower organic P concentrations (in mg P kg^{-1} of structural component) than those in productive pasture soils. At the same time, total and extractable inorganic P concentrations in bulk soil and structural components did not differ between pasture types, suggesting a shift from organic to non-extractable P upon degradation. Soils under productive pastures contained 37% more organic P than degraded pasture soils (in mg P kg^{-1} soil), mainly in large macroaggregates. The organic P concentrations were strongly correlated with C concentrations across all soil structural components, suggesting similar stabilization mechanisms for organic P and C. In bulk soil and most structural components, around 60% of organic P was enzyme-hydrolyzable. The lower contents of all enzyme-hydrolyzable as well as enzyme-stable organic P in degraded vs. productive pasture soils indicate a reduction of all organic P classes during pasture degradation. The large macroaggregates and, in particular, microaggregates occluded within this fraction were identified as an important site for organic P storage. Our results highlight ~~the~~ a linkage between soil structure and organic P, ~~which~~ both of which can ~~are~~ play an important role in ~~important in~~ maintaining the productivity of pastures established on highly weathered soils.

Keywords: Amazon Basin, Colombia, tropical pastures, pasture degradation, soil aggregation, phosphorus, phosphorus forms, enzyme additions

1. Introduction

Highly weathered soils of the humid tropics typically have low total and available phosphorus (P) contents and P is often the primary limiting nutrient to plant growth (Nziguheba and Bünemann, 2005; Vitousek et al., 2010). On deforested areas in South

America, pastures sown with introduced grasses (*Brachiaria* spp.) represent a dominant land use, and the majority of pastures exist in some stage of degradation (Jimenez and Lal, 2006). Pasture degradation is understood as a marked reduction in livestock production due to a significant decrease in plant biomass production and invasion of non-palatable plant species, and it leads to appearance of bare soil patches, soil compaction and reduced soil microbial biomass (Boddey et al., 2004; de Oliveira et al., 2004). Pasture degradation has enormous economic implications. For example, in Brazil every year about 8 million hectares of degraded pastures require considerable investment~~s~~ for renewal and/or recovery (Jank et al., 2014), with estimated costs of 100 to 200 US$ ~~/~~ha^{-1}, i.e., around 1 billion US$ in total (FAO, 2006). Soils of degraded pastures have been shown to store less carbon (C) than soils of productive pastures (Asner et al., 2004; Fonte et al., 2014). Pasture over-grazing and reduced pools of available nitrogen (N) and P are seen as the principal causes of degradation (Dias-Filho et al., 2001; Fisher et al., 2007). While the importance of N cycling and availability has been ~~shown~~ demonstrated previously (Boddey et al., 2004), a mechanistic understanding of the role of P in maintaining pasture productivity is missing.

Because most inorganic P is strongly sorbed, organic P has been suggested to play a critical role in sustaining P availability in highly weathered soils (Tiessen et al., 1984; Turner et al., 2006). Biological P cycling through plant uptake, residue decomposition and microbial turnover, which includes synthesis of organic P, ~~seems~~ appears to be crucial to protect P from sorption and to maintain P availability in highly weathered tropical soils (Oberson et al., 2006). Organic P constitutes 16% to 65% of total P in highly weathered soils (Nziguheba and Bünemann, 2005).

A single-step procedure involving sodium hydroxide and EDTA (ethylenediaminetetraacetate) is often used for the extraction of soil organic P from strongly weathered tropical soil (Turner, 2008). ~~and can be combined with enzyme a~~Additions of

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enzymes to various soil extracts have been used to characterize hydrolyzable forms of organic P, as reviewed by Bünemann (2008), e.g., addition of acid phosphatase or phytase to soil water extracts (Fox and Comerford, 1992; Shand and Smith, 1997). As different enzymes hydrolyze different organic P bonds, this approach delivers information on the chemical form of organic P in soils (He and Honeycutt, 2001; Turner et al., 2002a). Based on P release by acid phosphatase, phytase and nuclease from defined organic P compounds, Annaheim et al. (2013) classified the hydrolyzed P into simple monoesters, ~~nucleic acids and~~ *myo*-inositol hexakisphosphate and nucleic acids. Keller et al. (2012) used the same approach to classify organic P in NaOH-EDTH extracts. Enzyme-hydrolyzable organic P identified by phytase addition to EDTA extracts was shown to be ~~higher~~ greater in Oxisols under no till than with conventional tillage (Pavinato et al., 2010), suggesting that enzyme-hydrolyzable organic P is a sensitive indicator for the impacts of soil management on soil P status.

Soil aggregation is considered important for regulating the storage and turnover of soil organic C (Six et al. 2000) and nitrogen (N) (Bosshard et al., 2008). Relatively f~~F~~ew~~er~~ studies ~~concern~~have examined~~ed~~ the effect of aggregation on P and these have ~~shown~~ suggested that P availability, P concentrations and forms ~~are~~ can be influenced by aggregation (McDowell et al., 2007; Wang et al., 2001). For example, ~~Phosphorus~~P uptake was ~~higher~~ greater ~~by~~ in plants grown in large aggregates (2-6 mm) than in~~by~~ plants grown in small aggregates (< 0.5 mm) of highly weathered Oxisols because P bound to large aggregates was more readily desorbed (Wang et al., 2001). The water extractable P concentration decreased with smaller aggregate sizes in cropped soils in Canada (Messiga et al., 2011). The concentrations of orthophosphate, monoesters, diesters and pyrophosphates determined by ^{31}P-nuclear magnetic resonance (^{31}P-NMR) spectroscopy increased with decreasing aggregate size in a soil from New Zealand, while phosphonates and polyphosphates were unaffected (McDowell et al., 2007). We also note that t~~T~~he effect of management (or landuse) on P concentrations in different ~~a~~ structural components is amplified when~~if~~ ~~also~~ the proportion ~~mass fraction of the structural~~of these components on ~~the~~a whole soil mass basis is also affected.

In a recent study, the distribution of soil mass among aggregate size classes was found to differ between degraded and productive pasture soils (Fonte et al., 2014). Although soils of both pasture types had a high aggregate stability, the proportion of large macroaggregates (>2000 µm) was significantly higher in productive (65 g 100 g^{-1}) than in degraded pasture soils (56 g 100 g^{-1}). Soil of productive pastures had 20% higher total C and N contents (in g kg^{-1} soil) than degraded pastures. These differences in total soil organic matter (SOM) between pasture types were largely explained by a greater~~higher~~ C content in the large macroaggregate fraction, and more specifically in the microaggregates (53-250 µm) occluded within this macroaggregate fraction (~~mM~~Mmicros). Interestingly, there was no difference in total P content between pasture types, but organic P content was found to be nearly 40% greater~~higher~~ in soils of productive vs. degraded pastures. The findings of Fonte et al. (2014) suggest that different organic P contents in the bulk soils of productive and degraded pastures could be related to differences in soil structure and the distribution of C across~~in~~ aggregate fractions. Specifically, these results lead us to hypothesize that ~~higher~~ greater organic P content is associated with the greater protection of SOM in the ~~mM~~Mmicros fraction of productive pastures.

To further elucidate the role of aggregation in the P status of highly weathered tropical soils we examined the distribution of organic, inorganic and available P across aggregates and occluded macroaggregate fractions of the same degraded and productive pasture soils studied by Fonte et al. (2014). To obtain information on organic P forms, we determined enzyme-hydrolyzable organic P classes by enzyme additions. Finally, we studied the relationship between C and organic P across the different soil structural components.

2. Material and Methods

2.1 Site description, experimental design and soil sampling

The study was conducted on nine farms located in the deforested Amazon region of Colombia. All farms are situated within a 30 km radius of the city of Florencia, in the Department of Caquetá (1°36′50″N 75°36′46″W) with an average elevation of 280 m.a.s.l. The region has a humid tropical climate with a mean annual precipitation of 3400 mm and a mean annual temperature of 25°C. The mildly undulating topography is characterized by acid soils, mainly Oxisols and Ultisols (Mosquera et al., 2012) with textures in the 0-10 cm soil layer ranging from silty clay to sandy clay loam (Fonte et al., 2014).

The design of the study has been described in Fonte et al. (2014). Briefly, on each of the nine farms, areas of productive and degraded pasture were identified together with farmers. Degraded pastures had clear signs of degradation (e.g., patches of bare soil, invasion of herbaceous weeds and grasses other than the sown species, significant or total loss of previously sown *Brachiaria* spp.), while productive pastures were those dominated by *Brachiaria* spp. (mainly *B. humidicola* CIAT 679 and *B. decumbens* CIAT 606). Productive pastures had significantly ~~higher~~ greater live and litter biomass than degraded pastures (Table 1). Degraded and productive pastures had similar topography and age since pasture establishment. All farms experienced similar management histories, with forest cleared at least 40 years earlier. Basic soil properties are shown in *Table 1*.

~~Soils f~~For aggregate separation and P analyses, four soil sub-samples were taken ~~in each pasture type on all nine farms in July 2011~~ from regularly spaced sampling points, at 3 m from a central point in each pasture type on all nine farms in July 2011 ~~were sampled from the 0-10 cm soil layer in July 2011~~ (Fonte et al., 2014). ~~In brief, s~~Samples were carefully collected to a depth of 10 cm using a soil knife so as to incur minimum disruption of aggregates. The four sub-samples were combined, resulting in one sample per each pasture type per farm, or 18 samples in total. Field moist soil clods were gently broken along natural planes of weakness, so that they also passed the 8 mm sieve. Earthworms, termites, plant debris and stones were removed. The soil was dried at 50°C and shipped to the Soil Physics laboratory of the International Center for Tropical Agriculture (CIAT) in Cali, Colombia for aggregate separation.

2.2 Soil and macroaggregate fractionation

We analyzed sub-samples of the various aggregate size classes and occluded macroaggregate fractions isolated by Fonte et al. (2014). We here summarize the fractionation method. Soil samples were first wet-sieved based on Elliott et al. (1986) into large macroaggregates (> 2000 µm), small macroaggregates (250-2000 µm), microaggregates (53-250 µm) and the silt&clay (< 53 µm) fraction (Fig. 1). To this end, 80 g of soil were submerged in deionized water on a 2000 µm sieve and slaked for 5 minutes. The soil was then sieved by oscillating the sieve in and out of the water 50 times over a period of 2 minutes. Macroaggregates remaining on the sieve were then washed into a pre-weighed aluminium pan for drying. Soil which passed through the sieve was transferred to a smaller sieve and the sieving process repeated with a 250 µm and with a 53 µm sieve. All free aggregate size classes were dried in the oven at 50°C and their mass determined. The silt&clay fraction passing through the 53 µm sieve was associated with a large volume of water (approx. 3 l) and the slurry was dried together in a large pan. Before further processing, stones and organic material > 2 mm, which are not typically incorporated into soil aggregates, were removed and weighed.

Macroaggregates were further fractionated after the method of Six et al. (2000a) to obtain occluded fractions within both large and small macroaggregates (Fig. 1). Briefly, 6 g of macroaggregates were submerged in water for 24 h and kept at 4°C for slaking.

Macroaggregates were then placed on a modified 250 µm sieve, submerged in water and shaken horizontally (250 rpm) with 40 stainless steel beads (6 mm diameter) until all macroaggregates were broken up (3-8 min). While macroaggregates were being ruptured, particles smaller than 250 µm were transported by a constant water flow to a 53 µm sieve below. These particles were then wet-sieved as described above to separate microaggregates within macroaggregates (~~mM~~Mmicros) from silt&clay occluded in macroaggregates (~~Msc~~Msilt&clay). The fraction remaining on the 250 µm sieve constituted the coarse particulate organic matter (cPOM) and sand fraction. As for the free aggregate classes, all macroaggregate occluded fractions were dried in the oven at 50°C. A subsample was taken from bulk soil, aggregates and occluded fractions and ground to a powder using a ball mill (Retsch, GmbH, Germany) prior to analysis.

2.3 Nutrient analyses and enzyme addition assay

Concentrations of total P in bulk soil, aggregates and occluded fractions were measured colorimetrically (Ohno and Zibilske, 1991) after heat digestion of 200 mg sample with H_2O_2, H_2SO_4, Se, and Li_2O_4S at 340°C for 2 h 30 min (Anderson and Ingram, 1993). Plant available P was extracted from 1 g of sample using anion-exchange resin strips saturated with HCO_3^- after Kouno et al. (1995). Organic P was extracted from 500 mg of sample with 5 ml NaOH-EDTA solution (Bowman and Moir, 1993). Inorganic P in the NaOH-EDTA extracts was measured colorimetrically (Ohno and Zibilske, 1991). Another subsample of the extract was digested to obtain NaOH-EDTA total P by autoclaving the extract with H_2SO_4 and ammonium persulphate, subsequent neutralization and colorimetric measurement. Organic P was calculated as the difference between total and inorganic P in the NaOH-EDTA extract. The difference between total soil P (obtained with the H_2O_2-H_2SO_4 digestion described above) and NaOH-EDTA total P is non-extractable P.

The following enzymes were added to the NaOH-EDTA extracts in a microplate assay (Keller et al., 2012): (1) ~~Phytase from *Peniophora lycii* (RONOZYME® NP (M), DSM Nutritional Products Denmark), (2) ~~Acid phosphatase (EC 3.1.3.2) from potato, Sigma Chemicals, Product No. P1146, ~~and ~~(2~~3~~) Nuclease P1, from *Penicillium citrinum*, Sigma Chemicals, Product No. N8630 together with acid phosphatase, and (3) Phytase from *Peniophora lycii* (RONOZYME® NP (M), DSM Nutritional Products Denmark). After adding the enzymes and MES buffer (adjusted to a pH of 5.2), the microplate was incubated at 37°C for 48 h, followed by colorimetric determination of molybdate reactive P concentrations (Ohno and Zibilske, 1991) in enzyme-amended and non-amended extracts. Mean concentrations were calculated from four analytical replicates. The concentration of P released by a given enzyme was calculated as the difference in P measured in soil extracts incubated with and without the enzyme preparation, additionally accounting for the background concentration of molybdate reactive P in the enzyme preparation. This background was significant only for the phytase preparation. The differences were also corrected for P spike recoveries measured in each treatment, which were usually greater than 95%. Enzyme-hydrolyzable organic P was classified based on the enzyme specificity determinations of Annaheim et al. (2013):

(1) Simple monoester-like organic P: P in simple monoesters like glycerol phosphate, glucose-6-phosphate, in anhydrides and in condensed organic compounds like pyrophosphate and ATP; *P released by acid phosphatase.*

(2) DNA-like organic P: P in nucleic acids; *(P released by nuclease plus acid phosphatase)* – *(P released by acid phosphatase).*

(3) InosP-like organic P: P in *myo*-inositol hexakisphosphate; *(P released by phytase)* – *(P released by acid phosphatase).*

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The sum of all three enzyme-hydrolyzable P classes is the enzyme-hydrolyzable organic P. Enzyme-stable organic P in NaOH-EDTA extracts was calculated as the difference between organic P and enzyme-hydrolyzable organic P.

The P contents (mg P kg^{-1} soil) in the soil structural components were obtained by multiplying the P concentration of each component (mg kg^{-1}) by its mass (kg component kg^{-1} soil). The analyses were conducted on the nine replicate samples of bulk soil and structural components per pasture type. Exceptions due to limited sample mass are given in footnotes of tables and figures. The determinations of total, organic and available P were repeated at least three times for each sample, and the average of the three analytical replicates used. The sum of P extracted from the aggregate size classes (large and small macroaggregates, free microaggregates, free silt&clay) had the following average (± standard deviations) percentages of P extracted from the bulk soil: 101±12% for total P; 104±23% for available P; 100±21% for organic P; and 108±24% for enzyme-hydrolyzable organic P. The sum of P forms extracted from the fractions occluded in large and small macroaggregates, in percentage of total P of intact large and small macroaggregates, was on average 93±10% for total P, 105±23% for organic P, and 97±23% for enzyme-hydrolyzable organic P and 55±15% for available P. The recovery of available P does not include cPOM, as it could not be analyzed due to insufficient sample.

2.4 Statistical analysis

The statistical analyses were completed with R 2.12.2 (© 2011 The R Foundation for Statistical Computing). Comparisons and other calculations were carried out using mean values of the analytical replicates. All data was tested for normal distribution by the Shapiro normality test and transformed using natural log or arcsin when necessary. Differences between degraded and productive pastures were analysed using a linear mixed model with farms considered as blocks and treated as random variable. Likewise, ~~D~~differences between aggregates and fractions within pasture type were analysed using the same model ~~, and moreover between degraded and productive pastures,~~ since the interaction between pasture types and aggregates was not significant.~~were analyzed using a linear mixed model with farms considered as blocks and treated as random variable.~~ Soil texture (clay + silt) was included as a covariate in the linear mixed model since texture has been shown to have a significant influence on soil aggregation (Fonte et al., 2009), P concentration (McGrath et al., 2001) and SOM content (Feller and Beare, 1997). Significance level for all statistical tests was set at ~~P~~*p* = 0.05, unless reported otherwise.

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3. Results

3.1 Phosphorus concentrations in bulk soil, aggregates and fractions

Concentrations of total and available P in the bulk soil highlight the low P status of the soils, with average total P concentrations of 362 mg P kg^{-1} and less than 7 mg kg^{-1} of available P extracted using anion exchange resins (Table 2). Organic P concentration was significantly (37%) higher in the bulk soil of productive vs. degraded pasture soils, while total and available P concentrations were similar. Organic P constituted on average 19% of total P in degraded and 26% in productive pasture soils. On average 58% of the organic P was enzyme-hydrolyzable and simple-monoester-like P was the greatest identified organic P class in the bulk soil (Fig. 2). While there were hardly any significant differences between degraded and productive pasture soils in identified organic P classes, degraded pasture soils had significantly lower enzyme-stable organic P concentrations (Table 3).

Inorganic P and available P concentrations decreased with decreasing aggregate size classes while total and non-extractable P concentrations did not significantly vary across aggregate size classes (Table 2). Organic P was lowest in free microaggregates and highest in silt&clay. The total of enzyme-hydrolyzable organic P did not significantly change with aggregate size class (Table 2), but the concentrations of InosP-like P were ~~significantly~~ highest in the small macroaggregates, and in productive pasture~~s~~ soils also the enzyme-stable organic P concentrations were significantly affected by aggregation (Table 3). Total, inorganic and available P concentrations did not significantly differ between aggregate size classes of productive vs. degraded pasture soils (Table 2). In contrast, the organic P concentrations were significantly higher in the large and small macroaggregates and in the free microaggregates of the productive than in those of the degraded pasture soils. Macroaggregates from productive pasture soils also had significantly ~~higher~~ greater enzyme-stable organic P concentrations and InosP-like P was higher in free microaggregates from productive pastures~~,~~ while concentrations of other identified organic P forms were little affected by pasture type (Table 3).

Within macroaggregates, available P concentrations were higher in the occluded microaggregates than in the silt&clay (Table 4). Inorganic P, ~~O~~organic P and enzyme-hydrolyzable organic P concentrations were ~~significantly~~ lowest in the cPOM. Accordingly, concentrations of identified organic P forms were ~~significantly~~ lowest in cPOM (supplementary Fig. 1). At the same time, cPOM was characterized by the highest proportion of enzyme-hydrolyzable P (Fig. 2). Similar to the findings for macroaggregates, the pasture type also affected the P concentrations of the occluded fractions (Table 4). The available P concentrations were significantly lower in the ~~mM~~Mmicros and in the ~~Msc~~Msilt&clay fractions of small macroaggregates of the degraded than the productive pastures. Likewise, organic P concentrations were lower in the ~~mM~~Mmicros of large macroaggregates of degraded pastures. These differences were largely reflected in the enzyme-hydrolyzable organic P. All identified P forms, ~~but~~except for enzyme-stable organic P, showed the same characteristic of lower P concentrations in the Mmicros ~~of macroaggregates~~ of degraded than of productive pastures.

3.2 Phosphorus content in aggregates

Soil structure significantly differed between productive and degraded pasture soils (Fig. 3.1), as shown by Fonte et al. (2014). Differences in aggregate distribution and P concentrations resulted in significantly different contents of P forms, on a mg per kg^{-1} soil basis, between productive and degraded pastures (Fig. 3.2-3.6). Inorganic, organic and enzyme-hydrolyzable organic P contents were significantly ~~higher~~ greater in large macroaggregates of productive than degraded pastures. In contrast, the total, inorganic and available P contents were significantly ~~higher~~ greater in free microaggregates and silt&clay of degraded than productive pastures (inorganic P only in the microaggregates), but these are small pools due to their low overall proportion of the total soil mass ~~fraction~~ (Fig. 3.1).

Productive pasture soils contained significantly more enzyme-stable organic P and tended to have more InosP-like P than degraded pasture soils (Fig. 4). These differences were for several organic P forms significant in the large macroaggregates and the ~~mM~~Mmicros and ~~Msc~~Msilt&clay fractions, and were consistent with the overall ~~higher~~ greater organic P and enzyme-hydrolyzable organic P in these fractions (Fig. 3, 4).

Contents of all the P forms in aggregates and fractions decreased in an order similar to the one of the distribution of their mass fraction (Fig. 3.1). Thus, the ~~mM~~Mmicros of large and small macroaggregates had highest contents of all P pools while cPOM, free microaggregates and free silt&clay presented small pools (Fig. 3.2-3.6).

3.3 Relationship between carbon and organic P

Organic P and C concentrations were highly correlated across all structural soil components (Fig. 5, with C concentrations from Fonte et al. (2014)). As correlation coefficients and regression functions were similar for degraded and productive pasture soils, Fig. 5 includes data from both pasture types. It shows that concentrations of C and organic P changed in parallel across aggregate size and fraction. We note that t~~T~~he C and P concentrations for~~of~~ one cPOM sample were several times higher than that observed in ~~of~~ all other cPOM samples because of its lower sand content. The C to organic P ratio varied between 240 (silt&clay) and 890 (cPOM) (supplementary Table 1). It tended to decrease with aggregate size and was lowest in the silt&clay fraction. The C to organic P ratios were not significantly different between productive and degraded pasture soils.

4. Discussion

4.1 Aggregation affects organic and inorganic P contents

Our results suggest that soil aggregation is crucial in maintaining organic P contents in tropical grassland soils. In particular, most organic P was stored within~~ the~~ large macroaggregates, and more specifically within ~~them, mostly in the ~~microaggregates occluded within macroaggregates (~~mM~~Mmicros, Fig. 3.4). Since the concentrations of enzyme-hydrolyzable organic P largely changed in parallel with organic P across aggregates and occluded fractions, the ~~mM~~Mmicros fraction was also an important site for the storage of enzyme-hydrolyzable P forms and of enzyme-stable P (Fig. 4). McDowell et al. (2007) also found greater concentrations of organic P forms in aggregate size classes similar to our macroaggregates, than in unsieved soils or aggregates < 250 µm of a cultivated soil from New Zealand. However, these macroaggregates were not fractionated further for P analysis.

The distribution of organic P among aggregates and fractions was similar to the distribution previously observed for C (Fonte et al., 2014). Also, Denef et al. (2007) identified the ~~mM~~Mmicros to be a preferential site of C accumulation in Oxisols. Likewise, we found this same fraction to be important for organic P storage. The organic P concentration was significantly higher in both ~~mM~~Mmicros of large and small macroaggregates than in the free microaggregates, for degraded and productive pastures.

Aggregate formation and related physical protection of SOM within aggregates are largely driven by biotic processes (Six et al., 2000a; Tisdall and Oades, 1982). However, in highly weathered tropical soils dominated by 1:1 clays and Fe and Al (hydr)oxides, sorption of SOM to mineral particles provides an additional, important stabilization mechanism (Dungait et al., 2012; Six et al., 2000b). These particles can also strongly sorb organic P forms (Ognalaga et al., 1994; Pavinato et al., 2010). The strong correlation between C and organic P suggests that soil aggregation affects the stabilization of C and organic P to a similar degree (Fig. 5). Likewise, the decrease of the C to organic P ratios with aggregate size (supplementary Table 1) was in line with the decrease in the C to N ratios in the same aggregates (Fonte et al., 2014). Decreasing C to N ratios have been interpreted as more processed forms of SOM in aggregates of smaller size (Six et al., 2001).

Aggregation also significantly affected the concentration and contents of inorganic P, with inorganic P concentrations significantly decreasing with aggregate size (Table 2). Similarly, macroaggregates had greater concentrations of water extractable and/or EDTA extractable molybdate reactive P than microaggregates and the silt&clay sized particles in cropped Ultisols (Green et al., 2006). Due to highest concentration and proportion of total soil mass, the macroaggregates in our study contained a substantial amount of the NaOH-EDTA extractable inorganic P (Fig. 3.3). As for organic P, the occluded microaggregates were an important storage site and had higher inorganic P concentrations than the free microaggregates ($p < 0.001$). Likewise, they were a principal storage site of available P (Fig. 3.5). The importance of aggregation was ~~additionally~~ also indicated by the higher available P concentrations in the occluded than in the free microaggregates or the silt&clay fraction. This suggests that upon disaggregation, reactive sorption sites increase P sorption, which is in line with the stronger sorption on silt&clay reported earlier by Wang et al. (2001). Likewise, sorption was strongly increased following the destruction of organic matter by a H_2O_2 treatment of highly weathered soils dominated by 1:1 clays, as this organic matter had probably masked sorptive surfaces through organo-mineral aggregation (Frossard et al., 1992). Apparently, the increase in P sorption upon disaggregation also occured during the analyses of our study, as the sum of available P extracted from fractions occluded in large and small macroaggregates was only 55±14% of the available P extracted from the intact macroaggregates. Still, this sum does not include the cPOM, which could not be analyzed for available P due to insufficient sample.

4.2 Aggregation affects organic P forms

In the bulk soil and all structural components except the cPOM, about 60% of the extracted organic P was hydrolyzed by the added enzymes. This was a higher proportion than the 33-39% found in cropped temperate zone soils (Keller et al., 2012), but similar to the average of 58% of enzyme-hydrolyzable organic P in NaOH-EDTA extracts from soils of different climatic zones and land use (Jarosch et al., 2014). The high proportion of enzyme-hydrolyzable organic P supports the overall good physical protection of SOM and organic P in the studied soils, as described above and in Fonte et al. (2014).

Because inositol hexakisphosphates are stabilized through strong complexation with metals and clay surfaces (Celi and Barberis, 2005), they are often the dominant class of organic P in soils (Turner et al., 2002b) and we expected to see that confirmed in our study. InosP-like P was also the most abundant identified organic P class in enzyme addition studies on cropped and permanent grassland soils from the temperate zone (He et al., 2004; Keller et al., 2012). However, in our study the organic P contained in the soils and their structural components was not dominated by InosP (7-37% of organic P, Fig. 2). Our results corroborate Turner et al. (2007) who found, along a 120,000-year postglacial chronosequence in New Zealand, that proportions of organic P classes change with soil age, such that *myo*-inositol hexakisphosphate declines with increasing soil age and that inositol phosphates and other monoesters have similar concentrations in highly weathered soils. In another study using ^{31}P-NMR spectroscopy on NaOH-EDTA extracts, even no inositol phosphates were detected in an Oxisol under forest (Vincent et al., 2010).

We also expected that the concentrations as well as the proportions of organic P classes would differ between structural components due to aggregation-related changes in physico-chemical stabilization. Indeed, the concentrations and proportions of DNA-like P increased with decreasing aggregate size, which agrees with findings of McDowell et al. (2007) for diesters. This could be due to adsorption of DNA onto clays (Greaves and Wilson, 1969) or to microorganisms associated with the clay particles (van Gestel et al., 1996). On the other hand, apart from the greater concentration of InosP in the small macroaggregates, Inos-P and simple monoester-like P changed little with aggregation. This might be because also simple monoesters like glucose-1-phosphate get readily sorbed on Fe oxides such as goethite (Ognalaga et al., 1994). Still, the relatively high proportions of simple monoester like P might be somewhat overestimated due to the degradation of some orthophosphate diesters in studies involving alkaline extraction (Turner et al., 2003b). RNA and some phospholipids (phosphatidyl choline) were rapidly degraded during alkaline extraction and analysis (Makarov et al., 2002; Turner et al., 2003a). In contrast, DNA, other phospholipids, and orthophosphate monoesters were more stable (Turner et al., 2003a).

Our aim was to characterize total organic P using NaOH-EDTA extraction in combination with enzyme additions. The observed small differences in enzyme-hydrolyzable

P between structural components do not preclude that differences in enzyme-hydrolyzable P would be seen in milder extracts, such as citrate (Hayes et al., 2000), water or bicarbonate (George et al., 2007). Such milder extractants can only be used in soils in which they extract sufficient P to observe an increase in molybdate-reactive P following enzyme addition.

About 40% of the extracted organic P was not hydrolyzed by the added enzymes and its form therefore remains unidentified. Keller et al. (2012) suggested that this organic P might correspond to the broad signal found in the monoester region of ^{31}P-NMR spectra (Doolette and Smernik, 2011), which might be due to P associated with large humic compounds (Doolette et al., 2011). Because of incorporation into humic substances, it may not be accessible to enzymatic hydrolysis as shown for organic P incorporated into model humic polymers (Brannon and Sommers, 1985). ~~Indications on organic P associated with high-molecular weight polymeric substances, which is not enzyme-hydrolyzable, also comes from Pant et al. (1994) who applied molecular fractionation and enzyme assays to soil water extracts.~~

Contrary to our expectations, the proportion of enzyme-hydrolyzable organic P was similar in all structural components except the cPOM which had a significantly higher proportion of enzyme-hydrolyzable organic P forms. ~~Similar proportions of enzyme-hydrolyzable P, along with specific P forms largely changing in parallel with organic P concentrations, suggest similar processes of organic P synthesis and/or stabilization across the different aggregate fractions.~~ The cPOM~~, however,~~ consists mainly of relatively undecomposed plant debris (von Lützow et al., 2007) and P from fungi decomposing these plant residues (Salas et al., 2003), which explains that organic P contained therein will more readily undergo enzymatic hydrolysis. The more labile character of cPOM was furthermore indicated by the faster turnover time of C contained in cPOM than of C contained in any other structural component, as derived from the δ^{13}C signatures (Fonte et al., 2014). In contrast, the lower and similar proportions of enzyme-hydrolyzable P in all other structural components were in line with more similar C turnover times among these fractions.

Nevertheless, the pasture type affected the concentrations and contents of enzyme-hydrolyzable and enzyme-stable organic P forms in soils and several structural components. This indicates that all organic P forms underwent transformations during pasture degradation.

4.3 Impact of pasture degradation on P forms and content is related to soil structure decline

Comparison of the degraded and productive pasture soils illustrates the linkage between soil structure, P status and the implications for P dynamcis. Fonte et al. (2014) found ~~higher~~ greater contents of organic P in the bulk soils of productive vs. degraded pastures, despite a lack of differences in total P content, and suggested that this is related to differences in soil structure. More specifically, their findings suggested that ~~higher~~ greater organic P content is associated with the greater protection of SOM in the ~~mM~~Mmicros fraction of productive pastures. The P analyses presented here support this hypothesis.

Soils of productive pastures contained more large macroaggregates than degraded pasture soils (Fig. 3.1) (Fonte et al., 2014). Together with higher organic P concentrations in this fraction for productive vs. degraded pastures, this translated into ~~higher~~ greater organic P storage in the large macroaggregates of productive pastures. The organic P concentrations were higher in several other structural components of productive vs. degraded pasture soils as well, and the C and organic P concentrations were strongly linked across all structural components (Fig. 5). The loss of C (Asner et al., 2004; Fonte et al., 2014) and organic P from degraded pasture soils appear to be linked, as the C to organic P ratios were similar for degraded and productive pasture soils across all structural components and the bulk soil (supplementary Table 1). Concomitant loss of C and organic P was earlier reported for cultivated soils in semi-arid northeastern Brazil (Tiessen et al., 1992) and in North American prairie soils (Tiessen et al., 1982).

Several mechanisms could contribute to lower organic P contents in degraded than productive pasture soils, which are i) ~~lower~~ reduc~~d~~ed organic inputs, ii) less organic P synthesis due to lower microbial activity, ~~and, last but not least~~ iii) loss of organic P due to reduced physical protection of SOM in aggregates and iv) due to prolonged anoxic soil conditions. These mechanisms are interlinked, and are discussed in detail below.

The input of C and organic P contained in aboveground plant litter (Table 1) and in belowground residues, ~~(~~because of lower root density (Fonte et al., 2014),~~)~~ was lower in degraded than in productive pastures. Furthermore, due to the lower amount of forage offered by degraded pastures, less ~~organic P~~ C and organic P in animal manure were~~was~~ recycled back to the soil. ~~via animal manure.~~

Carbon input stimulates microbial growth and activity (Bünemann et al., 2004a). Microbial growth is related to microbial P uptake, which involved moderately available soil P in highly weathered tropical soils (Bünemann et al., 2004b), and which is related to organic P synthesis (Bünemann et al., 2008). Soil organic P has been suggested to be largely of microbial origin (Magid et al., 1996). Thus, the lower microbial activity in degraded vs. productive pasture soils ~~(e.g.,~~ as suggested by lower microbial respiration (~~;~~ Table 1), could result in lower organic P synthesis.

Aggregate formation is largely driven by biotic processes (Six et al., 2000a; Tisdall and Oades, 1982), such as the activity of plant roots, microorganisms and earthworms (Fonte et al., 2012; Guggenberger et al., 1999). Since organic inputs drive soil microbial and faunal activity (Bünemann et al., 2004a; Lavelle et al., 2001), lower inputs in degraded pastures likely result in lower soil aggregation and, in consequence, lower physical protection of SOM, including organic P. The lower organic P contents at similar total P contents indicate that due to lower protection, this P was mineralized. Because several soil structural components of degraded pastures contained less extractable inorganic and/or available P than in productive pasture soils, at similar total P concentrations, the mineralized organic P was most probably converted into non-extractable P forms (Tables 2, 4). Conversion of mineralized organic P into non-extractable P has been reported in earlier studies on tropical soils, e.g., from Brazil, where SOM degradation following the cultivation of a forest soil transformed organic P into non-extractable forms (Tiessen et al., 1992). This process was irreversible, despite fallow periods and a return of SOM to former levels (Tiessen et al., 1992). We have not studied the reversibility of organic P decline in our study, but we observed a similar organic P decline as Tiessen et al. (1992) (37% in our study vs. 30%). Thus, inappropriate tropical pasture management appears to have resulted in similar organic P losses to those observed with cultivation.

Degraded pastures soils are often compacted (Boddey et al., 2004), as illustrated by the greater values of bulk density of degraded vs. productive pasture soils studied here (Table 1). Compacted soils may undergo longer phases of waterlogging than non-compacted soils. Similar to observations for inorganic P (Schärer et al., 2009), prolonged anoxic conditions associated with compaction could increase the desorption of organic compounds (Peretyazhko and Sposito, 2005), making them more suceptible to enzymatic attack and also potentially more prone to losses. Concentrations of dissolved inorganic and organic P were sometimes greater in river waters from pasture-dominated as compared to forested watersheds in the Brazilian Amazon (Neill et al., 2001), suggesting that pastures lose more P than forests. However, Neill et al. (2001) did not investigate the status of the pastures, and the significance of specific nutrient loss pathways such as surface runoff, erosion and leaching remains unknown. Considering the similar total P contents of degraded and productive pastures soils, we have no indication that there was a net loss of P from degraded pastures in our study. This also suggests that there was no net P loss via grazing animals which could be explained by mineral P supplementation to grazing cattle (Dias-Filho et al., 2001).

The decline of organic P associated with degradation affected all organic P forms. This suggests that physical protection in aggregates is an important mechanism of organic P stabilization, as for overall SOM (Denef et al., 2007; Six et al., 2000a).

~~*4.4 Other processes affecting P dynamics in pasture soils*~~

~~The low P availability in pasture soils probably induced plant strategies to mobilize P. *Brachiaria* grasses were shown to increase oxalic acid exudation, root acid phosphatase and phytase activities in response to P deficiency (Louw-Gaume et al., 2010). The secretion of organic anions from *Nicotiana tabacum* was shown to release inositiol phosphates from goethite and render them accessible to phytase (Giles et al., 2012). Still, this mobilization and utilization of organic P would have retained P in the biological P cycle, as defined earlier.~~

~~Degraded pastures soils are often compacted (Boddey et al., 2004), as illustrated by the greater values of bulk density of degraded vs. productive pasture soils studied here (Table 1). Compacted soils could~~ may ~~undergo longer phases of waterlogging than non-compacted soils. Similar to observations for inorganic P (Schärer et al., 2009), prolonged anoxic conditions associated with compaction could increase the desorption of organic compounds (Peretyazhko and Sposito, 2005), making them more suceptible to enzymatic attack and also potentially more prone to losses. Concentrations of dissolved inorganic and organic P were sometimes higher~~ greater ~~in river waters from pasture-dominated as compared to forested watersheds in the Brazilian Amazon (Neill et al., 2001), suggesting that pastures lose more P than forests. However, Neill et al. (2001) did not investigate the status of the pastures, and the significance of specific nutrient loss pathways such as surface runoff, erosion and leaching remains unknown. Considering the similar total P contents of degraded and productive~~

~~pastures soils, we have no indication that there was a net loss of P from degraded pastures in our study. Likewise, we have no indication on net P loss by P export via grazing animals. Estimated P exports via live weight gain of grazing animals from productive *Brachiaria decumbens* pastures in the Colombian Llanos were 1.6 kg P ha^{-1} year^{-1} (Oberson et al., 1999). This P may be replenished through the mineral P supplementation to grazing cattle (Dias-Filho et al., 2001).~~

~~The available P measured here by anion exchange resins differed little between degraded and productive pasture soils. Resin extractable P is a snapshot measurement with great temporal variation previously observed for tropical pasture soils in Colombia (Oberson et al., 1999). Available P is defined as the amount of P that can replenish the soil solution and be taken up during the time span relevant for plant growth (Frossard et al., 2000). The replenishment of the soil solution is mediated by abiotic and biotic processes from inorganic and organic P forms. Our results suggest reduced capacity of the degraded soil to deliver available P from each of these processes. Measurements of microbial turnover and organic mineralization in incubation studies (Bünemann et al., 2012; Oberson et al., 2001) or *in situ* field measurements of available soil P fluxes by anion exchange resin probes (Meason and Idol, 2008) or diffusive gradients in thin films (DGT) (Six et al., 2012) might contribute to further understand the differences between degraded and productive pastures soils.~~

5. Conclusions

This study shows a clear linkage between soil structure and organic P. ~~which is important in maintaining the productivity of pastures established on highly weathered soils.~~ Large macroaggregates, and in particular the microaggregates occluded within macroaggregates, were identified as an important site of organic P storage. Soils under degraded pastures ~~contained less organic P because of less organic inputs and reduced soil~~

~~aggregation. Specifically, they~~ contained fewer large macroaggregates in which C and organic P can be physically protected. The observed reduction of organic P, in turn, affected all identified enzyme-hydrolyzable P forms as well as enzyme-stable P.

Degraded and productive pasture soils and their structural components had similar total P concentrations. Thus, we have no indication that soil P depletion was causing pasture degradation. However, the supply of plant available P could be lower in degraded than productive pasture soils because of reduced biological P cycling and because of increased P sorption due to ~~less~~ reduced aggregation. ~~intact~~

About 60% of the extracted organic P was hydrolyzed by the added enzymes. Contrary to our expectations, this proportion was similar in all soil structural components, except in the cPOM. This is the first time that enzymatic organic P characterization has been applied to NaOH-EDTA extracts obtained from aggregate size classes and their occluded fractions. Since highly weathered tropical soils are specific in their mineralogy, similar studies on temperate zone soils are needed to understand the effect of soil structure on organic forms and their hydrolyzability.

~~Our findings suggest that the efficient use of P in highly weathered tropical soils requires the maintenance of soil structure. Sustainable pasture management requires grazing management which prevents physical soil damage by overgrazing and which sustains organic matter return to stimulate overall soil biological activity and nutrient cycling. Restoring degraded pasture soils requires reconstructing soil structure through enhanced biological activity and may require improved forage or crop components with specific P mobilization mechanisms to bring recalcitrant P back into the cycle.~~

Our findings suggest that sustaining P availability in highly weathered tropical soils requires the maintenance of intact soil structure. Sustainable pasture management needs grazing management that prevents physical damage to soils and sustains organic matter return to stimulate overall soil biological activity and nutrient cycling. Restoration of degraded pasture soils requires reformation of soil structure through enhanced biological activity and may need improved forage or crop components that stimulate specific P mobilization mechanisms to bring recalcitrant P back into the cycle.

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Figure legends

Fig. 1. Bulk soil separation into large macroaggregates, small macroaggregates, microaggregates and silt&clay after Elliott (1986) and subsequent fractionation of macroaggregates into coarse particulate organic matter and sand (cPOM), microaggregates within macroaggregates (~~mM~~Mmicros) and occluded silt&clay (~~Msc~~Msilt&clay) after Six et al. (2000a).

Fig. 2. Proportion of enzyme-hydrolyzable P forms and enzyme-stable P in organic P of the bulk soil, the aggregate size classes and the occluded macroaggregate fractions. Macros = macroaggregates, D = degraded pastures, P = productive pastures. For acronyms refer to the caption of Fig.1. Means and standard errors from n = 9, except for i) silt&clay (n = 6 (degraded) and n = 4 (productive)), ii) cPOM (n = 3) and ~~Msc~~Msilt&clay of large macroaggregates (n = 7 (degraded)), iii) cPOM (n = 4) and ~~Msc~~Msilt&clay of small macroaggregates (n = 7 (degraded) and n = 8 (productive)).

Fig. 3. 1) Mass distribution of aggregates and occluded fractions, contents of 2) total P, 3) inorganic P, 4) organic P, 5) available P and of 6) enzyme-hydrolyzable organic P, in aggregate size classes and occluded fractions, in mg kg^{-1}soil. Black outlines are contents in aggregate size classes and filled bars are sums of occluded macroaggregate fractions. For acronyms see caption of Fig.1, D = degraded pastures, P = productive pastures. Means of n = 9. Different letters between two bars denote significant differences ($p < 0.05$) or a trend ($p <$ 0.1) between pasture types, with letters outside the bars being assigned to aggregate size

classes, and letters inside bars being assigned to occluded macroaggregate fractions. Error bars denote standard error of content in aggregate size classes.

Fig. 4. Contents of simple monoester-, DNA- and InosP-like organic P in aggregate size classes and occluded macroaggregate fractions, in mg P kg^{-1} soil. For acronyms see caption of Fig.1, D = degraded pastures, P = productive pastures. Means from n = 9 except for cPOM (n = 3) and silt&clay (n = 7 (degraded)) of large macroaggregates, cPOM (n = 3) and silt&clay (n = 7 (degraded) and n = 8 (productive)) of small macroaggregates. Different letters between two bars denote significant differences ($p < 0.05$) or a trend ($p < 0.1$) between pasture types, with letters outside the bars being assigned to aggregate size classes, and letters inside bars being assigned to occluded macroaggregate fractions. Black outlines are contents in aggregate size classes and filled bars are contents of summed occluded macroaggregate fractions. Error bars denote standard error of content in aggregate size classes.

Supplementary Fig. 1. Concentrations of specific P forms in the occluded macroaggregate fractions, in mg kg^{-1} fraction. Different lower case letters denote significant differences ($p < 0.05$) between pasture types. Different capital letters indicate significant differences between the fractions.

Table 1 Bulk soil characteristics (0-10 cm) and aboveground plant biomass.

Pasture	C [a]	N [a]	Clay [b]	Silt [b]	Bulk density	pH[c]	Al[d]	Basal soil respiration[e]	Live aboveground biomass[f]	Litter biomass[f]
	mg kg^{-1} soil		g 100g^{-1} soil		g dry soil cm^{-3}		%	mg CO_2 kg^{-1} 45d^{-1}	--- g m^{-2} ---	
Degraded	23 (1.4) a	2.0 (0.1) a	44 (2)	32 (5)	1.24 (0.02) b	4.34 (0.03) a	81 (2)	253 (35) a	47 (5) a	136 (24) a
Productive	28 (2.1) b	2.4 (0.1) b	42 (2)	38 (4)	1.13 (0.03) a	4.50 (0.06) b	71 (7)	486 (60) b	101 (16) b	380 (66) b

Means and standard error in brackets, with n = 9 except for bulk density (n = 7). Different lower case letters denote significant differences ($p < 0.05$) between pasture types.

[a] C and N concentrations were determined with a ThermoFisher Flash-Elemental Analyzer 1112 (EA) (Fonte et al., 2014).

[b] Soil texture was analysed after Bouyoucos (1962).

[c] pH measured in deionized water.

[d] Al saturation (%) = Al (cmol (+) kg^{-1} dry soil) * 100 / (Ca (cmol (+) kg^{-1} dry soil) + Mg (cmol (+) kg^{-1} dry soil) + K (cmol (+) kg^{-1} dry soil) + Al (cmol (+) kg^{-1} dry soil).

[e] Basal soil respiration analysis after Alef (1995) (n=7 (degraded) and n=6 (productive pastures)).

[f] From Fonte et al. (2014).

Table 2 Phosphorus concentrations in bulk soil and aggregate size classes.

Phosphorus forms		Bulk soil		Large macroaggregates		Small macroaggregates		Microaggreates		Silt&clay	
		mg P kg^{-1} soil		---------- mg P kg^{-1} aggregate ----------							
Total P [a]	Degraded	362 (54)		399 (57)		329 (42)		335 (56)		334 (62)	
	Productive	362 (38)		377 (32)	B†	351 (17)	AB	307 (28)	A†	329 (54)	AB
Available P [b]	Degraded	5.9 (0.4)		6.8 (0.7)	B	5.2 (0.5)	a† AB	4.4 (0.5)	A	4.7 (0.9)	A
	Productive	6.6 (0.6)		7.1 (0.6)	B	6.8 (0.5)	b† B	5.2 (0.5)	B†	3.4 (1)	A†
Inorganic P [c]	Degraded	51 (8)		61 (8)	C	51 (6)	a† BC	42 (7)	AB	35 (7)	A
	Productive	57 (6)		67 (9)	C	60 (5)	b† BC	45 (4)	AB	39 (10)	A
Organic P [c]	Degraded	68 (10)	a	68 (10)	a AB	70 (12)	a AB	62 (11)	a A	90 (14)	B
	Productive	93 (8)	b	92 (11)	b AB	99 (9)	b B†	76 (6)	b A†	105 (15)	B
Enzyme-hydrolyzable organic P [d]	Degraded	41 (6)	a†	42 (6)	a†	48 (6)		41 (9)	a	46 (7)	a†
	Productive	52 (4)	b†	52 (6)	b†	58 (4)		54 (5)	b	50 (3)	b†
Non-extractable P [e]	Degraded	244 (38)		271 (40)		208 (27)		231 (39)		210 (43)	
	Productive	212 (26)		218 (17)		192 (11)		186 (19)		184 (30)	
Microbial P [f]	Degraded	25 (3)		n.d.		n.d.		n.d.		n.d.	
	Productive	31 (3)		n.d.		n.d.		n.d.		n.d.	

Means and standard error in brackets, with n = 9, except for microbial P (n = 7), and enzyme-hydrolyzable organic P in the silt&clay fraction (n = 6 (degraded), n = 4 (productive pastures)). Different lower case letters denote significant differences ($p < 0.05$) or a trend† ($p < 0.1$) between pasture types. Different capital letters indicate in each row significantly different concentrations between aggregate size classes; n.d. = not determined

[a] Extracted by heat digestion with H_2O_2, H_2SO_4, Se, and Li_2O_4S (Anderson and Ingram, 1993).

[b] Extracted using anion exchange membranes (Kouno et al., 1995).

[c] Extracted using NaOH-EDTA (Bowman and Moir, 1993).

[d] Enzyme assay applied to NaOH-EDTA extracts (Keller et al., 2012).

[e] Difference between total soil P (obtained with the H_2O_2-H_2SO_4 digestion) and NaOH-EDTA total P.

[f] Microbial P analyzed after Kouno et al. (1995).

Table 3 Speciation of enzyme-hydrolyzable organic P in bulk soil and aggregate size classes.

Organic P forms	Pasture type	Bulk soil	Large macroaggregates	Small macroaggregates	Microaggregates	Silt&clay
		mg P kg^{-1} soil	--mg P kg^{-1} aggregate---			
Monoester-like	Degraded	19.5 (1.9)	17.1 (2.6)	12.9 (2.2)	15.9 (2.1)	18.8 (4.5)
	Productive	23.4 (3.1)	22.3 (4.0)	17.3 (3.3)	17.2 (2.4)	15.7 (0.7)
DNA-like	Degraded	11.7 (1.8)	12.7 (3.9) a† A†	13.1 (3.8) A†	12.6 (8.9) a† A	18.7 (2.1) B†
	Productive	13.9 (2.0)	14.7 (1.3) b†	15.8 (1.3)	17.9 (3.0) b†	19.4 (1.5)
InosP-like	Degraded	9.7 (2.7) a†	12.3 (2.9) A	22.3 (3.7) B	12.9 (5.1) a A	8.6 (1.0) a† A
	Productive	14.2 (1.7) b†	15.3 (2.3) A	25.4 (2.1) B	19.1 (2.2) b A	14.5 (2.8) b† A
Enzyme-stable	Degraded	26.6 (3.9) a	26.2 (4.8) a	22.1 (7.4) a	21.1 (3.4)	30.6 (6.9)
	Productive	41.8 (7.1) b	39.7 (6.3) bB	40.2 (8.6) bB	22.1 (4.0) A	36.2 (3.7) AB

Means and standard error in brackets, with n = 9 except for silt&clay (n = 6 (degraded) and n = 4 (productive pastures)). Different lower case letters denote significant differences ($p < 0.05$) or a trend† ($p < 0.1$) between pasture types. Different capital letters indicate in each row significantly different concentrations between aggregate size classes.

Table 4 Phosphorus concentrations in occluded macroaggregate fractions.

Phosphorus forms [a]		Large macroaggregates[a]						Small macroaggregates[a]					
		cPOM		Mmicros		Msilt&clay		cPOM		Mmicros		Msilt&clay	
		mg P kg^{-1} fraction											
Total P	Degraded	551 (174)		353 (51)		355 (58)		571 (228)		387 (48)		299 (40)	
	Productive	471 (89)		376 (41)		343 (45)		402 (102)		396 (40)		362 (54)	
Available P	Degraded	n.d.		5.4 (0.7)	C†	1.7 (0.4)	A	n.d.		4.2 (0.5)	aB†	1.2 (0.3)	aA
	Productive	n.d.		6.1 (0.9)	B	1.6 (0.1)	A	n.d.		5.5 (0.6)	bB	2.5 (0.5)	bA
Inorganic P	Degraded	14 (5)	A	65 (9)	B	47 (7)	B	27 (17)	A	61 (8)	B	47 (7)	B
	Productive	26 (10)	A	72 (10)	B	49 (8)	B	33 (12)	A	74 (12)	B	49 (7)	B
Organic P	Degraded	20 (3)	B	86 (12)	aC	100 (17)	C	11.4 (0.4)	A	80 (12)	C	81 (15)	C
	Productive	21 (1)	A	114 (14)	bC†	110 (20)	BC	60 (70)	AB†	103 (11)	BC	105 (19)	BC
Enzyme-hydrolyzable organic P	Degraded	15.8 (5.8)	A	56 (6)	a†B	50 (11)	a†B	9.5 (3.3)	A	42 (6)	aB	44 (4)	aB
	Productive	18.2 (0.2)	A	70 (12)	b†B	61 (11)	b†B	27.1 (17.2)	A	66 (8)	bB	61 (9)	bB
Non-extractable P	Degraded	111 (61)	A†	203 (32)	B†	208 (38)	B†	159 (118)	A	246 (30)	B	171 (28)	B†
	Productive	175 (127)		190 (20)		183 (19)		132 (176)		219 (21)		208 (29)	

Means and standard error in brackets, with n = 9 except for i) organic P, inorganic P, enzyme-hydrolyzable organic P and non-extractable P in cPOM in large macroaggregates (n = 3, with total P (mg kg^{-1}) = 144 for degraded and total P (mg kg^{-1}) = 222 for productive pastures), in cPOM in small macroaggregates (n = 4, total P (mg kg^{-1}) = 197 for degraded and total P (mg kg^{-1}) = 225 for productive pastures), ii) enzyme-hydrolyzable organic P in silt&clay of large macroaggregates (n = 7 (degraded pastures, with total P (mg kg^{-1}) = 375) as well as of small macroaggregates (n = 7 (degraded pastures, with total P (mg kg^{-1}) = 299), and n = 8 (productive pastures, total P (mg kg^{-1}) = 362). Different lower case letters denote significant differences ($p < 0.05$) and a trend† ($p < 0.1$) between

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pasture types. Different capital letters indicate in each row significantly different concentrations between the six occluded fractions; n.d. = not determined.

[a] For P forms see footnotes Table 2.

[b] For acronyms see caption of Fig.1.

Supplementary Table 1 Ratio of C to organic P in bulk soil and structural components.

	Pasture type			
	Degraded		Productive	
Bulk soil	375(45)		306 (24)	
Aggregate size classes				
Large macroaggregates	386 (44)	C B	320 (31)	A
Small macroaggregates	336 (39)	AB†	282 (25)	A
Microaggregates	319 (23)	A†	290 (21)	A
Silt&clay	286 (19)	A	240 (16)	A
Large macroaggregate occluded fractions [a]				
cPOM	545 (57)	BC	700 (126)	B
Mmicros	342 (34)	ABC	280 (16)	A
Msilt&clay	259 (20)	A	253 (14)	A
Small macroaggregate occluded fractions [a]				
cPOM	614 (207)	C	891 (328)	B
Mmicros	362 (41)	ABC	310 (18)	A
Msilt&clay	314 (27)	b†A†	257 (14)	a† A

Means and standard error in brackets, with n = 9 except for silt&clay (n=8 (productive pastures) and for cPOM in large macroaggregates (n= 3), cPOM in small macroaggregates (n = 4).

Different capital letters in a column denote significant differences ($p < 0.05$) and a trend† ($p < 0.1$) between structural components.

[a] For acronyms see caption of Fig.1.

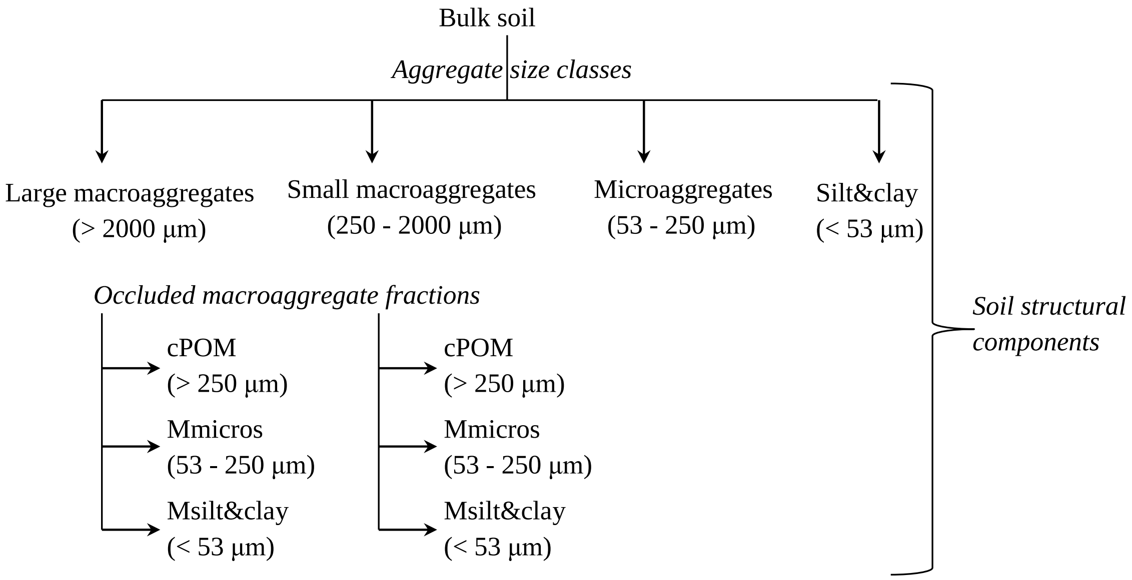

Bulk soil
Aggregate size classes
Large macroaggregates
(> 2000 µm)
Small macroaggregates
(250 - 2000 µm)
Microaggregates
(53 - 250 µm)
Silt&clay
(< 53 µm)
Occluded macroaggregate fractions
cPOM
(> 250 µm)
Mmicros
(53 - 250 µm)
Msilt&clay
(< 53 µm)
cPOM
(> 250 µm)
Mmicros
(53 - 250 µm)
Msilt&clay
(< 53 µm)
Soil structural
components

Fig. 1

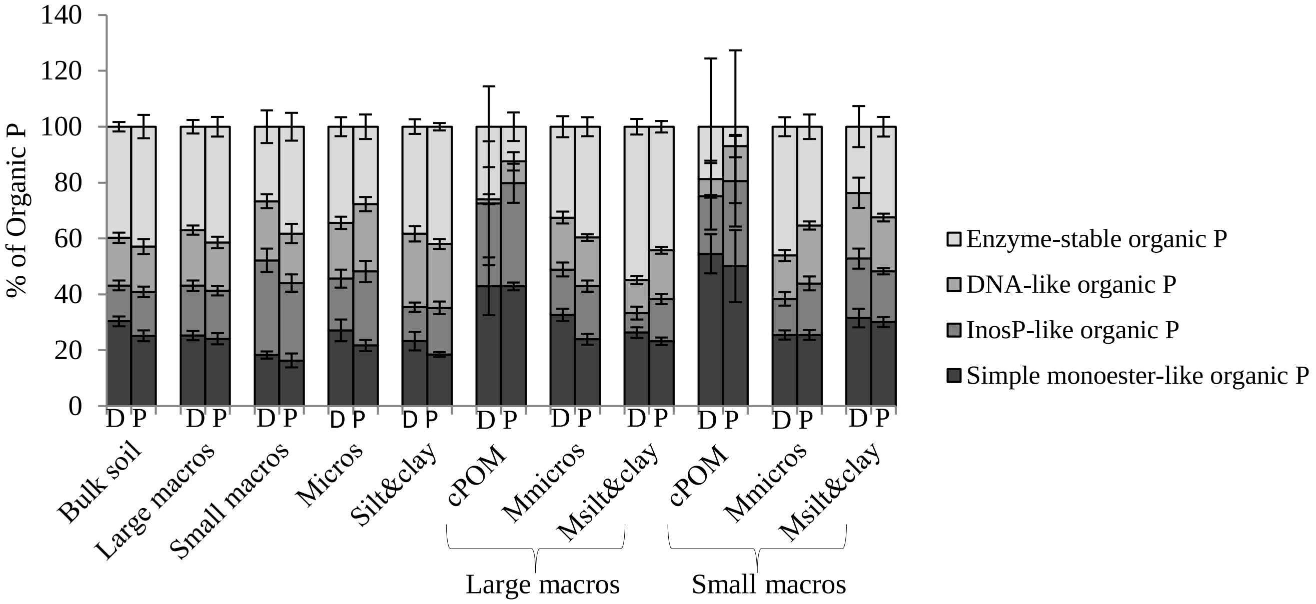

140
120
100
80
60
40
20
0
% of Organic P
D P
Bulk soil
Large macros
Small macros
Micros
Silt&clay
cPOM
Mmicros
Msilt&clay
cPOM
Mmicros
Msilt&clay
Large macros
Small macros
Enzyme-stable organic P
DNA-like organic P
InosP-like organic P
Simple monoester-like organic P

Fig. 2

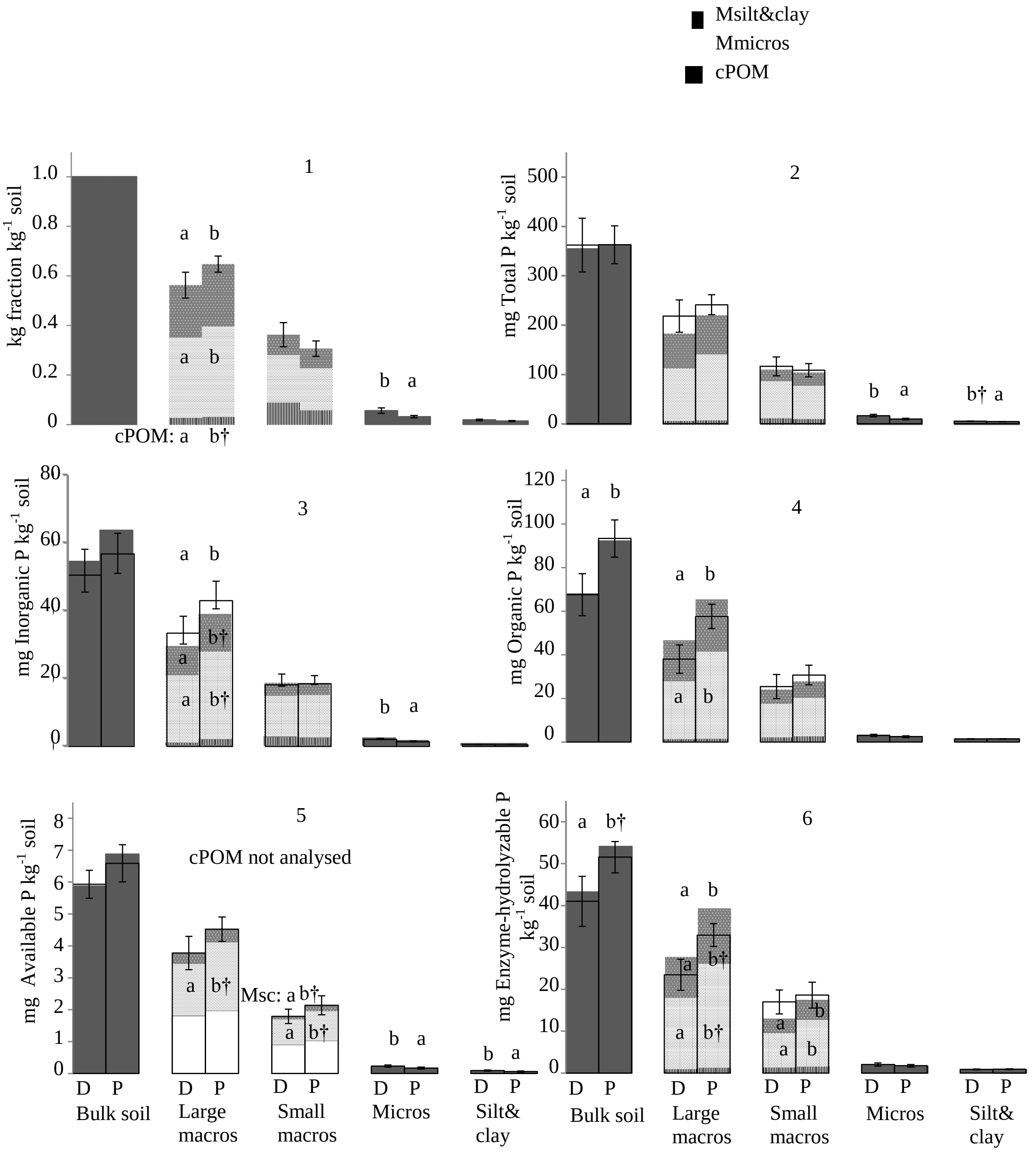

Msilt&clay
Mmicros
cPOM
1
kg fraction kg^{-1} soil
1.0
0.8
0.6
0.4
0.2
0
a b
a b
b a
cPOM: a b†
2
mg Total P kg^{-1} soil
500
400
300
200
100
0
b a
b† a
3
mg Inorganic P kg^{-1} soil
80
60
40
20
0
a b
a b†
a b†
b a
4
mg Organic P kg^{-1} soil
120
100
80
60
40
20
0
a b
a b
a b
5
mg Available P kg^{-1} soil
8
7
6
5
4
3
2
1
0
cPOM not analysed
a b†
Msc: a b†
a b†
b a
b a
6
mg Enzyme-hydrolyzable P kg^{-1} soil
60
50
40
30
20
10
0
a b†
a b
a b†
a b†
a b
a b
D P
Bulk soil
Large macros
Small macros
Micros
Silt& clay

Fig. 3

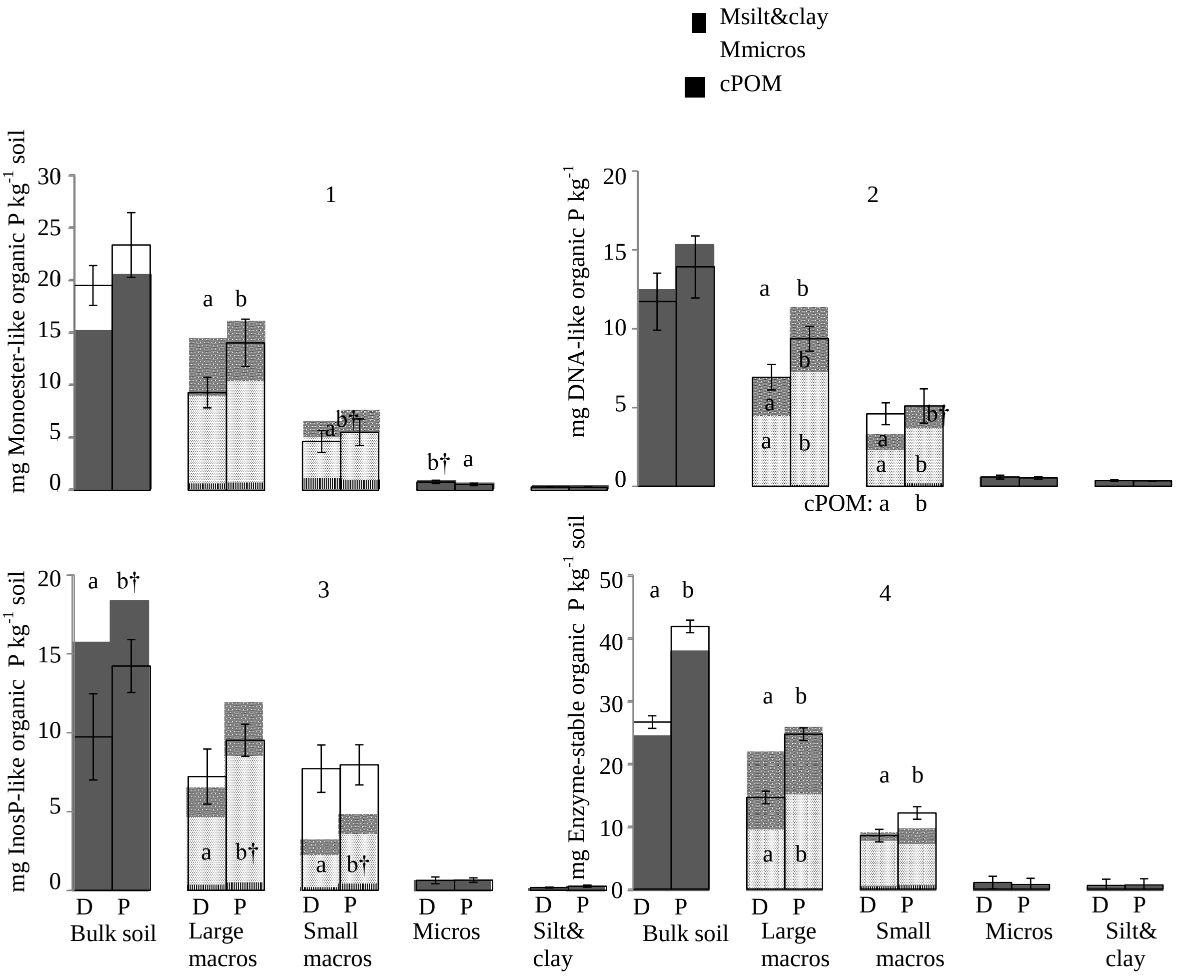

Msilt&clay
Mmicros
cPOM
1
mg Monoester-like organic P kg-1 soil
30
25
20
15
10
5
0
a
b
a
b†
b†
a
2
mg DNA-like organic P kg-1
20
15
10
5
0
a
b
a
b
a
b
a
b†
a
a
b
cPOM: a
b
3
mg InosP-like organic P kg-1 soil
20
15
10
5
0
a
b†
a
b†
a
b†
4
mg Enzyme-stable organic P kg-1 soil
50
40
30
20
10
0
a
b
a
b
a
b
a
b
D
P
Bulk soil
D
P
Large macros
D
P
Small macros
D
P
Micros
D
P
Silt& clay
D
P
Bulk soil
D
P
Large macros
D
P
Small macros
D
P
Micros
D
P
Silt& clay

Fig 4

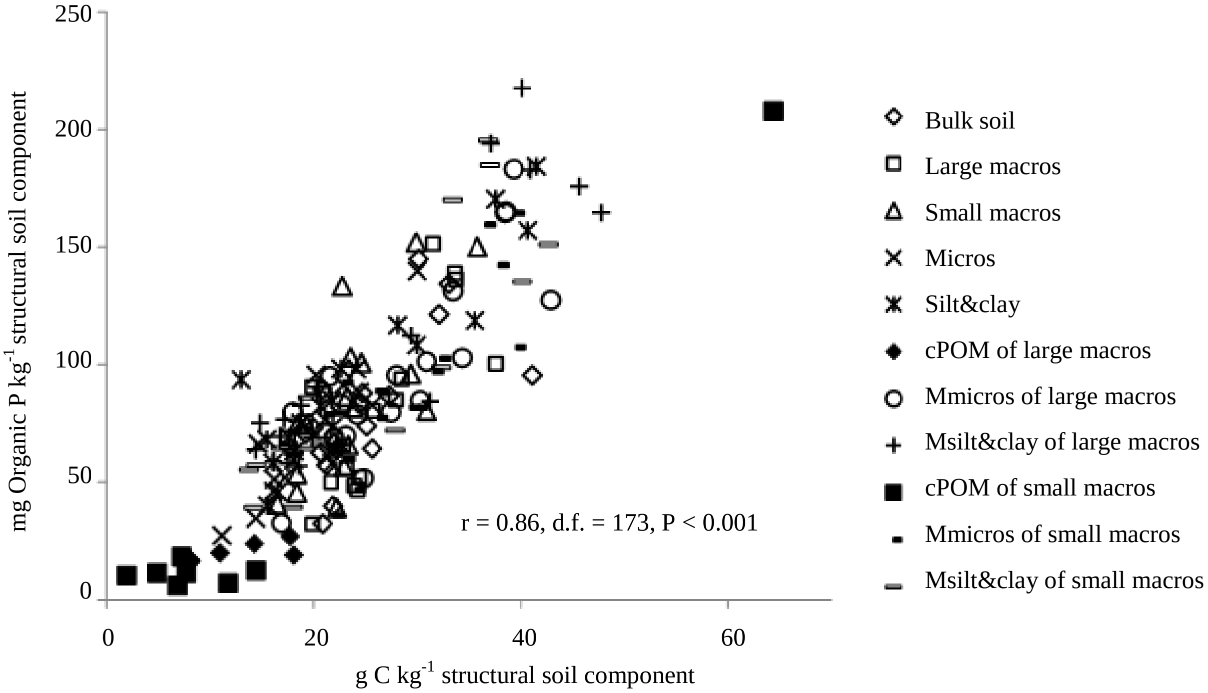
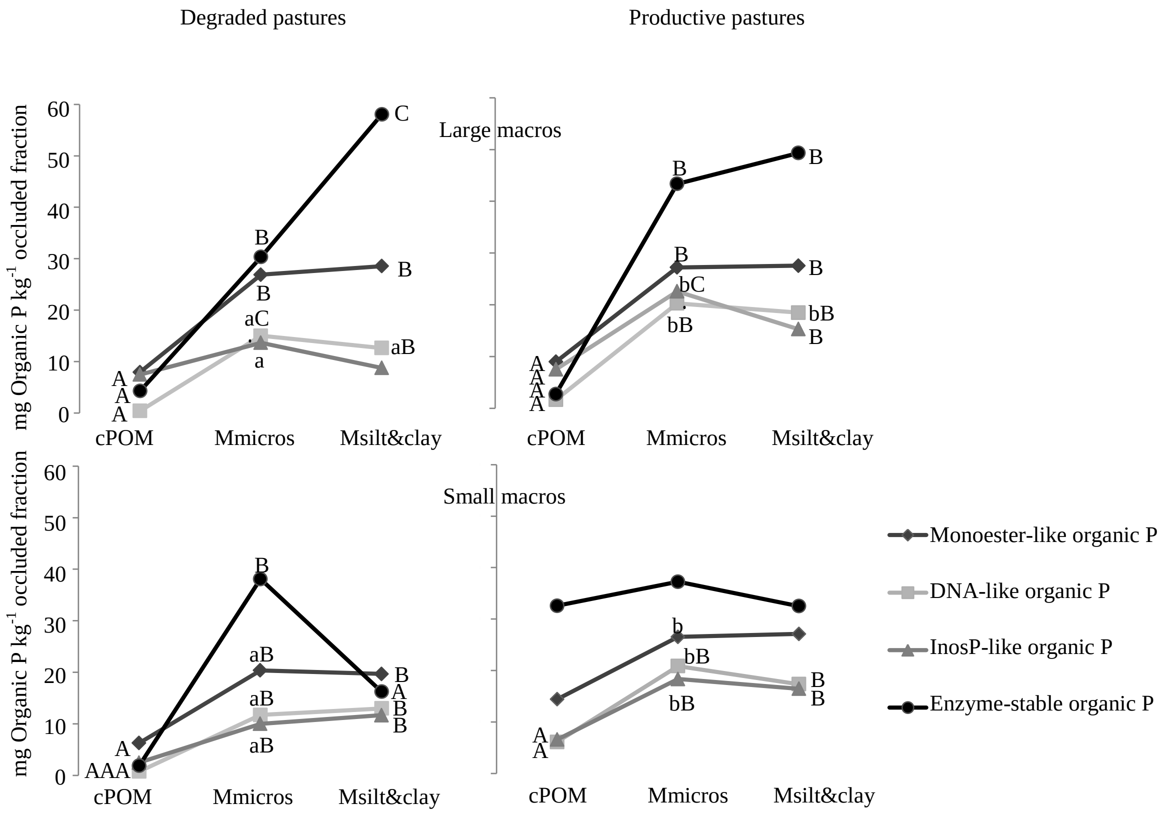

250
200
150
100
50
0
0
20
40
60
mg Organic P kg^{-1} structural soil component
g C kg^{-1} structural soil component
r = 0.86, d.f. = 173, P < 0.001
◇ Bulk soil
□ Large macros
△ Small macros
× Micros
✱ Silt&clay
◆ cPOM of large macros
○ Mmicros of large macros
+ Msilt&clay of large macros
■ cPOM of small macros
▬ Mmicros of small macros
▬ Msilt&clay of small macros

Fig. 5



Supplementary Fig. 1

***Responses to Technical Check Results**

Response to technical check results

We received no such comments and include therefore no such response file.