

# **Land-use Control on Abiotic and Biotic Mechanisms of P Mobilization**

Dissertation

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*To My Parents – Ricardo & Ma. Norma*

*To My Fiancè – Ranulfo Lumanao*

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## IV Abbreviations

AEM	Anion exchange membrane
Al	Aluminium
ANOVA	Analysis of Variance
C	Carbon
DFe <sup>2+</sup>	Dissolved Fe <sup>2+</sup>
DOC	Dissolved organic matter
DP	Dissolved phosphorus
Fe	Iron
MBC	Microbial biomass carbon
N	Nitrogen
P	Phosphorus
P <sub>i</sub>	Inorganic phosphorus
P <sub>o</sub>	Organic phosphorus
P <sub>AEM</sub>	Available phosphorus extracted by anion exchange membrane
P <sub>mic</sub>	Microbial biomass phosphorus
P <sub>NaHCO<sub>3</sub></sub>	Available phosphorus extracted by NaHCO <sub>3</sub>
P <sub>NaOH</sub>	Fe-bound phosphorus extracted by NaOH
P <sub>HCl</sub>	Non-available phosphorus extracted by HCl
SOM	Soil organic matter
TP	Total phosphorus
WHC	Water holding capacity

## V Summary

Phosphorus (P) is the most limiting nutrient for plant growth and productivity in many regions worldwide especially in the tropics. Aside intrinsic low P availability controlled by physicochemical and biological reactions, erosion and yield harvest are also very crucial in P depletion. These processes are massively intensified through anthropogenic activities, such as land-use change, the predominant global change of this century due to increasing population and food demand. Land-use change in consequence, affects P mobilization directly or indirectly through major modification of soil properties and functions. Hence, profound knowledge on abiotic and biotic factors affecting various P pools is necessary to understand the P dynamics and mobilization and to obtain a more effective soil management practices towards P conservation. Most studies were focused only on assessing the effects of land-use change on available P, but the other P pools such as Fe-bound P and microbial biomass P which are very important as reserve P pools especially in P-depleted soil were rarely considered. Therefore, this thesis aims at assessing the impacts of land-use on abiotic and biotic processes controlling forms, distribution and availability of P in soil.

The P sequential fractionation approach following Hedley method (1982) was used to assess the various P pools. The Hedley fractionation method estimates the P forms that have potential contribution to available P over a growing season. The extent of the method on extracting P from various pools and the mechanisms behind P dynamics was validated in an incubation experiment using  $^{33}\text{P}$  tracer isotope. The incorporation of  $^{33}\text{P}$ -labeled  $\text{KH}_2\text{PO}_4$  was traced in available P, microbial biomass P and Fe-bound P pools in an acidic P-depleted soil (Cambisol) depending on availability of carbon and nitrogen provided via applying glucose and ammonium sulfate, respectively. The Hedley fractionation was very efficient and accurate in extracting various P forms. The P immobilization via microbial uptake and fixation by the Fe and Al oxides was almost instantaneous. Applying glucose boosted microbial growth and so demand for P, resulting in increased  $^{33}\text{P}$  recovery and P content in microbial biomass. The microbial biomass P, as the most important labile P reservoir prohibits P fixation and increases the availability of P to plants during biomass turnover. In contrast, the high  $^{33}\text{P}$  recovery in Fe-bound P pool showed the dominance of P adsorption by Fe and Al oxides on P fixation and so less availability for plants.

The potential contribution of earthworms (another biotic factor) on P availability was also investigated. By coupling  $^{14}\text{C}$  imaging and direct zymography for the first time, we visualized and localized the effects of earthworms on distribution of litter and C compounds as well as enzymes activity throughout soil profile. Earthworms bury above ground litter, produce casts and mucus that enhance the activity of beneficial soil microorganisms, colonizing earthworms' biopores and so affect the P mobilization. Indeed, increase in microbial biomass P in the biopores and the activity of phosphatase enzymes which is responsible in hydrolyzing recalcitrant forms of organic P to become available for plants, were recorded.

In the second part of this thesis, we found out that the change of forests to: (a) intensively-managed oil palm and rubber plantations in the tropics and; (b) organic and conventional farming in sub-tropics alters the distribution of P pools through controlling abiotic and biotic reactions in soil. Organic and inorganic fertilizers application increases easily-available inorganic P. However, by decrease of easily-available organic P, moderately-available and non-available P intensifies. This means that fertilization maintains soil fertility only for a short time and fertilization is not sustainable in the long run due to the depletion of P reserves. The mechanisms of depletion in this easily-available P pool through land-use change are: 1) soil erosion; 2) microbial mineralization of soil organic matter (SOM) and 3) P export via yield products.

The intensified reduction in SOM contents induced by land-use change is the major influencing factor on P mobilization. Decreasing SOM furthermore, promotes soil compaction and reduces soil water holding capacity that leads to flooding. In the third part of this thesis, we demonstrated that anaerobic conditions which may take place following flooding accompanying decreasing SOM contribute to P mobilization and so the potential uptake of P by plant roots. The extent of microbial-mediated reduction process leading to dissolution of ferric oxides is apparently determined by the SOM content. SOM is the source of carbon and energy which enables microorganisms to efficiently reduce  $\text{Fe}^{3+}$ . Therefore, soils under forest and agroforest, with relatively high SOM content, resulted in a faster and higher P release than the plantation soils. Furthermore, increasing bulk density and in consequence flooding in soils under rubber and particularly under oil palm plantations led to lengthier anaerobic conditions and so more  $\text{Fe}^{3+}$  reduction and P release.

In conclusion, land-use change leads to major modification of soil properties and functions that affect abiotic and biotic mechanisms controlling the dominant type of P

pool and their distribution in a soil, and determine the dynamics of P pools transformation and P availability for plants. Among all the affecting factors, the mechanisms controlling P mobilization and availability are more closely linked to SOM content. Thus, ecologically-based managements to reduce SOM content loss are necessary to have the highest P availability for plants and so higher yield.

## Zusammenfassung

Phosphor (P) ist am meisten der einschränkende Nährstoff für Pflanzenwachstum und Produktivität in vielen Regionen der Welt, vor allem in den Tropen. Abgesehen von weniger P-Verfügbarkeit, die von den physikalischen, chemischen und biologischen Reaktionen dominiert wird, sind Bodenerosion und Ertragsernte bei der P-Erschöpfung sehr wichtig. Diese Prozesse werden durch die anthropogene Aktivitäten, wie die Landnutzungsumwandlung, die die vorherrschende globale Veränderung dieses Jahrhunderts ist - aufgrund der zunehmenden Bevölkerungsnachfrage nach Nahrung und anderen Produkten - massiv verändert. Dies führt zu einer wesentlichen Veränderung der Bodeneigenschaften und Funktionen, die direkt bzw. indirekt die P-Mobilisierung beeinflussen. Ein fundiertes Wissen über abiotische und biotische Faktoren, die verschiedene P-Pools beeinflussen, wäre für das Verständnis der P-Dynamik und Mobilisierung in den Böden sowie das Erhalten effektiverer Boden-Management-Praktiken in Richtung P-Erhaltung sehr wichtig. Die meisten Studien konzentrierten sich nur auf die Beurteilung der Auswirkungen der Landnutzung auf den labilen P-Pool, aber die anderen P-Pools, die als Reserve-Pool insbesondere in P-abgereicherten Böden sehr wichtig sind, werden selten berücksichtigt. Daher zielt diese Arbeit darauf ab, die Auswirkungen von Landnutzungsänderungen auf abiotische und biotische Prozesse, die die Formen, Verteilung und Verfügbarkeit der P im Boden kontrollieren, zu steuern.

Der P-sequentielle Fraktionierungsansatz nach der Hedley-Methode wurde verwendet, um die verschiedenen P-Pools in den Böden zu beurteilen. Die Hedley-Fraktionierungsmethode liefert die Schätzungen von verschiedenen P-Formen, die einen möglichen Beitrag zur verfügbaren P über eine Wachstumsphase haben. Das Ausmaß der Methode zur Extraktion von P aus verschiedenen Pools und den Mechanismen hinter der P-Dynamik wurde in einem Inkubationsexperiment mit  $^{33}\text{P}$ -isotopen Tracer validiert. Die Einführung der  $^{33}\text{P}$ -markierten  $\text{KH}_2\text{PO}_4$  wurde in den verfügbaren P-, mikrobiellen Biomasse-P- und Fe-gebundenen P-Pools in einem sauren P-abgereicherten Boden (Cambisol) in Abhängigkeit von der Verfügbarkeit von Kohlenstoff und Stickstoff, die durch Glucose bzw. Ammoniumsulfat angewendet wurden, verfolgt. Die Hedley-Fraktionierung erwies sich als sehr effizient und genau bei der Extraktion von verschiedenen P-Pools. Die P-Immobilisierung durch mikrobielle Aufnahme und die P-Fixierung durch die Fe- und Al-Oxide war fast augenblicklich. Die Anwendung von Glukose steigert das mikrobielle Wachstum und



die Nachfrage nach P, was zu einer erhöhten  $^{33}\text{P}$ -Rückgewinnung und einem P-Inhalt in mikrobieller Biomasse führt. Die P in mikrobielle Biomasse als das wichtigste labile P Pool verbietet die P-Fixierung und erhöht die Verfügbarkeit der P für die Pflanzen während des Biomasse Umsatzes. im Gegensatz zeigte die hohe  $^{33}\text{P}$ -Erholung im Fe-gebundenen P-Pool die Dominanz der P-Adsorption durch Fe- und Al-Oxide auf der P Fixierung und unavailability der P für die Pflanzen.

Der mögliche Beitrag der Regenwürmer (ein weiterer biotischer Faktor) zur P-Verfügbarkeit wurde ebenfalls untersucht. Durch Kopplung von  $^{14}\text{C}$ -Bildgebung und direkter Zymographie visualisierten und lokalisierten wir zum ersten Mal, die Wirkung von Regenwürmern auf die Verteilung von Pflanzenreste und C-Verbindungen sowie die Enzymaktivität im gesamten Bodenprofil. Regenwürmern begraben die über dem Boden liegenden organische Substanz und produzieren Guss und Schleim, die die Aktivität von nützlichen Bodenmikroorganismen, die in den Bioporen kolonisieren, verstärken und so beeinflussen die P-Mobilisierung. In der Tat wurde die Erhöhung der mikrobielle Biomasse P in den Bioporen und die Aktivität von Phosphatase-Enzymen, die bei der Hydrolyse der widerspenstigen Formen von organischem P verantwortlich sind, aufgezeichnet.

Im zweiten Teil dieser Arbeit haben wir herausgefunden, dass die Veränderung der Wälder zu: (a) intensiv verwalteten Ölpalmen und Kautschukplantagen in den Tropen und; (b) die organische und konventionelle Landwirtschaft in Subtropen verändert die Verteilung von P-Pools via kontrollieren der abiotische und biotische Reaktionen im Boden. Die organische und anorganische Düngemittelanwendung erhöht die labile anorganische P. Allerdings würde in kurzem die labile organische P vermindert und so die mäßig verfügbare und nicht verfügbare P intensiviert. Das heißt dass die Bodenfruchtbarkeit durch Landnutzungsintensivierung nur kurzfristig beibehalten würde und deshalb so eine Landnutzung ist nicht nachhaltig weil auf eine längere Zeit zu Erschöpfung der P-Reserven führt. Die Mechanismen dieser P Reserve Erschöpfung sind: 1) Bodenerosion; 2) mikrobielle Mineralisierung der organische Substanzen (SOM) und 3) P Export mit der Ertrag.

Die intensive Reduktion des SOM-Inhalts ist der wesentliche Einflussfaktor für die P-Mobilisierung. Außerdem führt die Erschöpfung von SOM zu die Bodenverdichtung und in Folge zu einer Überschwemmung. I dritter Teil dieses Studie haben wir gezeigt, dass Anaerober Zustand nachfolgend der Überschwemmungen und begleitet mit verringerter SOM-Inhalt zur P-Mobilisierung und damit zur möglichen Aufnahme durch Pflanzenwurzeln beigetragen haben. Das Ausmaß der mikrobiell

vermittelten reduktiven Auflösung von Eisenoxiden wurde offensichtlich durch den SOM-Inhalt bestimmt, da es sich um eine Kohlenstoff- und Energiequelle handelt, die es Mikroorganismen ermöglicht,  $\text{Fe}^{3+}$  effizient zu reduzieren. Böden unter dem Wald und Dschungelgummi, die die hohe SOM enthalten, führten zu einer schnelleren und höheren P-Freisetzung als die Plantagenböden. Außerdem leiden Gummi- und insbesondere Ölpalmenplantagen an einer Bodenverdichtung, was zu einer höheren Überschwemmungen und so längeren anaeroben Bedingungen führt.

Abschließend führt die Landnutzungsumwandlung zu einer starken Veränderung der Bodeneigenschaften und Funktionen, die der abiotische und biotische Mechanismen beeinflussen, die den Dynamik von P-Pools-Transformationen, und die pflanzliche Verfügbarkeit bestimmen. Unter allen Einflussfaktoren sind die Mechanismen, die P-Mobilisierung und Verfügbarkeit steuern, eng mit dem SOM-Inhalt verknüpft. Damit ist ein ökologisch fundiertes Management zur Reduzierung der SOM-Verlusten erforderlich um die hohe der P Verfügbarkeit und höhere Ertrag beibehalten.

## VI Acknowledgements

*Poverty is not a Hindrance to SUCCESS!  
To GOD be the GLORY!*

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# 1 Extended Summary

## 1.1 General Introduction

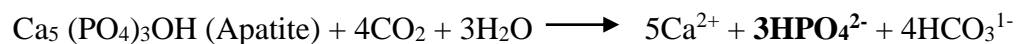
### 1.1.1 Role and relevance of P in plant nutrition

Phosphorus (P) is ubiquitous in nature (Chimdi et al., 2014). P, as orthophosphate, is essential to life, as a structural and functional component of all living organisms. It is present in a number of important plant cell compounds, such as sugar-phosphate, phospholipids in plant membranes, and nucleotides required for the accumulation and release of energy for cellular metabolism, as well as in control processes and in genetic material (Kirkby & Le Bot, 1994).

P is primarily absorbed by plant roots from soil solution as orthophosphate ions (principally dihydrogen phosphate,  $\text{H}_2\text{PO}_4^-$  and to a lesser extent  $\text{HPO}_4^{2-}$ ).

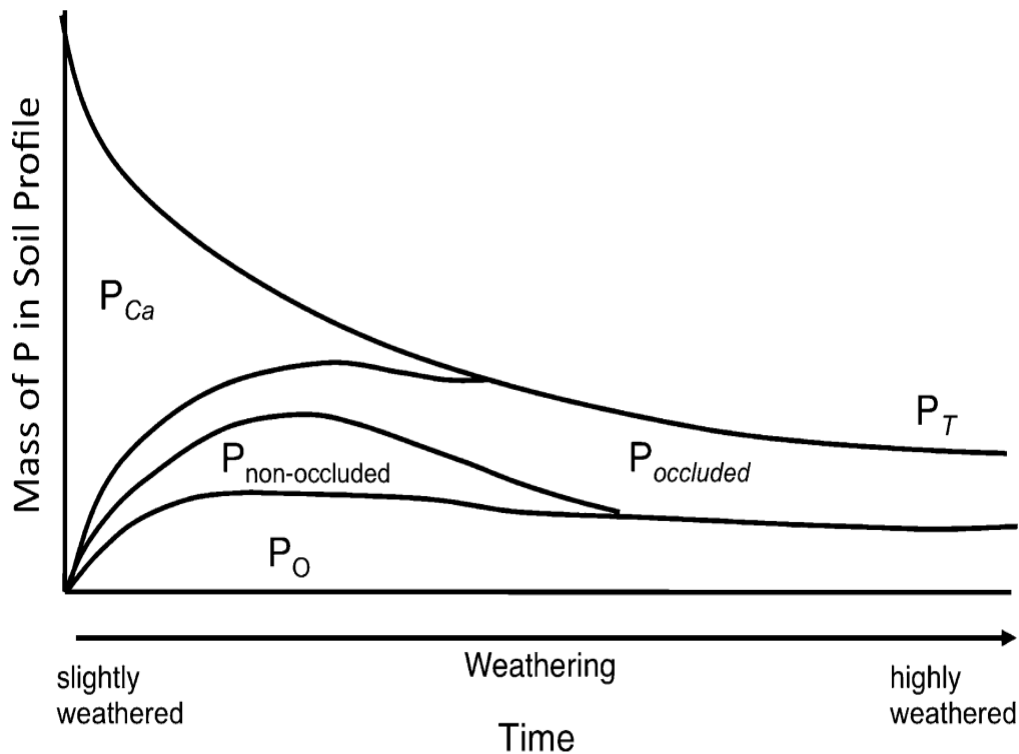
### 1.1.2 The P cycle in soil

According to Walker and Syers' conceptual model (1976) (Fig. S1), all soil P is in the primary form, mainly as Apatite, at the beginning of soil development. With time, the action of different factors (e.g., climate, slope, organisms) exerted on the parent material (Apatite) during the weathering process causes Apatitic P to solubilize: react with dissolved carbon dioxide and congruently release P:



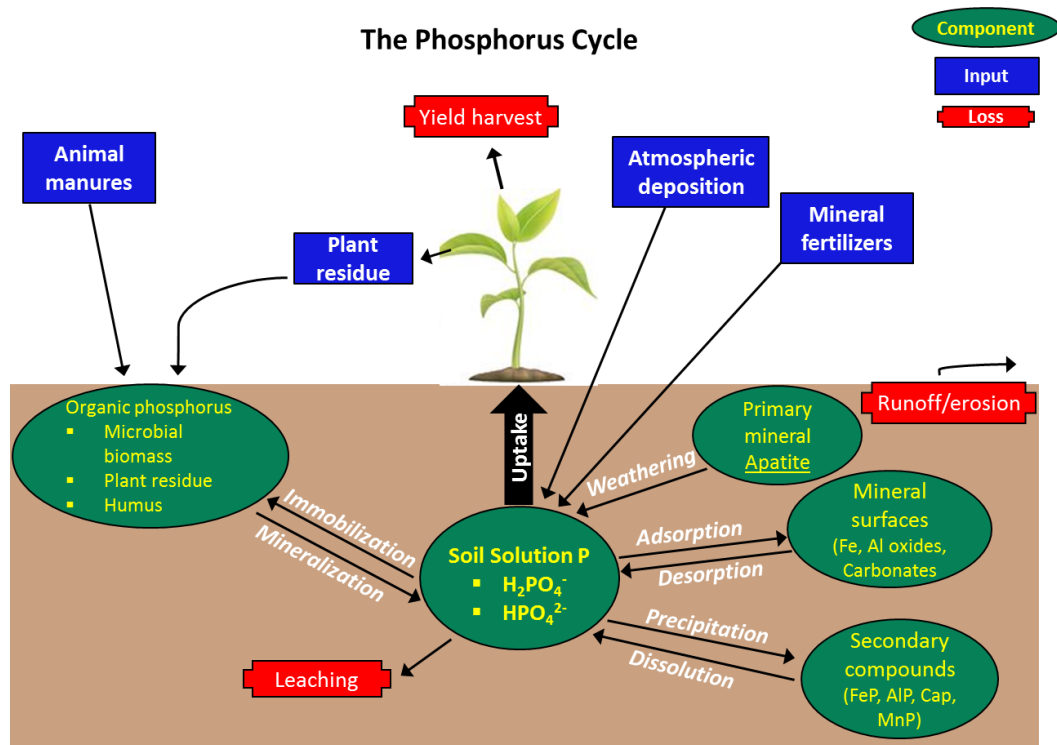
This give rise to P in various other forms, i.e., organic P (Po), non-occluded P and occluded P. The ionic form of solubilised P depends on the pH of the solution, with the predominate species in slightly acidic soils being  $\text{H}_2\text{PO}_4^-$  and in soils with a pH over 7 being  $\text{HPO}_4^{2-}$ . The liberated  $\text{PO}_4^{3-}$  can be taken up by plants and microorganisms entering the organic P reservoir, or ultimately returned to inorganic P (Pi) pools in the soil via mineralization. Nonetheless, during each turn of this cycle, some P may also be sorbed onto the surface of Fe and Al oxides and mineral edges of clay particles to become "non-

occluded P". With time, the non-occluded P is slowly encapsulated physically or surrounded by secondary minerals, which will continually be converted to occluded P. Therefore, at the late stage of soil development, soil P is dominated by organic P and occluded P. Furthermore, a decline of total P due to leaching, erosion and yield harvest could occur, consequently depleting available P for plant growth and productivity.



**Figure S1** Walker and Syers' (1976) conceptual model of soil P geochemistry transformation over time. The model shows the transformation of mineral phosphorus into non-occluded and organic forms before eventual dominance of occluded (oxide-bound) and organic forms. The relative bio-reactivity of phosphorus increases from mineral to occluded to organic forms of phosphorus. Note the continual loss of total phosphorus from the system.

In general, the P transformation processes controlling the P cycle in soil includes: (1) dissolution and precipitation; (2) immobilization and mineralization; (3) adsorption and desorption; (4) leaching and erosion. Figure S2 shows the different pathways of P in ecosystems, including fertilizer application and yield harvest.



**Figure S2** Phosphorus dynamics in an agricultural system. Adapted from: Potash & Phosphate Institute, (PPI), Georgia, USA.

### 1.1.3 Soil P forms and availability

Soil P can be grouped according to form and availability. P can be inorganic (Pi) or organic (Po) and of different availability and chemical bindings (i.e. available, moderately available, non-available inorganic and organic) (Hedley et al., 1982). With regards to availability and chemical bindings, P can be grouped into refractory (non-available) and labile (readily-available). The refractory forms are more stable in nature and include P in apatite minerals and P co-precipitated with and/or adsorbed by Fe, Al and Mn (hydro)oxides (termed “occluded” P). The importance of refractory forms has been frequently reported (Neufeldt et al., 2000; Reddy et al., 1999; Sharpley, 1985; Tiessen et al., 1984; Zheng et al., 2002). Depending on soil type and management, non-available P forms can be mobilized and become available for plant uptake, with mobilization rates depending on P form, desorption-status, weathering, and mineralization processes. On the other hand, labile forms include soil Pi that moves readily among plants, soil biota, soil solution (termed “available” Pi), loosely bound Pi (termed “non-occluded” or “moderately-available” Pi), organic pools incorporated in soil organic matter

(SOM) (termed “easily-mineralized” Po) and P pools associated with microbial biomass (termed “Pmic”). Easily-mineralized Po and Pmic are very important P reserve pools involved in soil P transformation and P cycling when soil Pi reserves are limited (Buehler et al., 2002). The distribution of P between these forms changes dramatically with time and soil development, as discussed earlier (*section 1.1.2*). When available P is depleted, replenishment from other P forms becomes important (Henriquez, 2002). Syers (1976) showed that the proportion of Po increases at the later stages of soil development, especially in relation to labile Pi. Thus, the relative contribution of soil biological processes to delivering plant-available P may become more important when the availability of Pi is low.

### *1.1.4 Mechanisms controlling P availability in soils*

P dynamics and availability are characterized by physicochemical (sorption-desorption) and biological processes (immobilization-mineralization) that are prevalent in soils and involve changes in various P pools. Despite the fact that these processes occur naturally, they are greatly modified by human activities (Beauchemin & Simard, 2000; Reddy et al., 1999). At the beginning of soil development, the availability of P in the ecosystem is restricted by the rate weathering-induced release from the parent material. As soil development progresses, changes in physicochemical and biological reactions and processes mediated by anthropogenic activities control the fate of various P pools.

#### *1.1.4.1 Abiotic control: P sorption, anaerobic condition, soil organic matter*

P sorption, which includes both adsorption and precipitation, is a very important process controlling P mobilization in the soil. Both Po and Pi are susceptible to sorption, but the extent is influenced primarily by the concentration, chemistry and solubility of soil P (Berg and Joern, 2006). P sorption reactions are particularly important in highly weathered soils because strong soil sorption capacities compete with biological sinks for P, effectively reducing P availability (Uehara and Gillman, 1980; Sollins et al., 1988). In highly weathered soils, 1:1 clays (e.g., kaolinite) and Al and Fe hydr(oxides) that effectively sorb P are prevalent. Consequently, sorbed P concentrations

often exceed those of soil solution by several orders of magnitude (Sanchez 1976). Fertilization studies have shown that, in highly weathered soils, P may be rapidly and nearly completely sorbed over short timescales (e.g., Sanchez, 1976; Uehara and Gillman, 1980; Oberson et al., 2001). Nonetheless, occluded P may actually enter available P pools, which are influenced by biological demand (Tiessen et al., 1984; Olander and Vitousek, 2004; Richter et al., 2006). For example, the increase in biological P in Piedmont forest regrowth (>28 yrs) came at the expense of P residing in the Fe-, Al- and occluded-P pools. This suggests that plants may have access to these pools over decadal (or perhaps shorter) timescales (Richter et al., 2006). Therefore, desorption of fixed P sustains long-term P fertility. In addition, P sorption is very important for keeping P in the soil system, as it protects P reserves from leaching and surface run-off.

Recent studies show that the interactions between soil solution P and Fe oxides are more temporally dynamic than previously thought, and are strongly influenced by soil oxidation states (Baldwin and Mitchell, 2000; Liptzin and Silver, 2009). In many tropical forests, for example, abundant rainfall coupled with high biological activity promotes episodic anoxia (Silver et al., 1999; Schuur and Matson, 2001). Consequently, the corresponding fluctuations in redox potential can release Fe-bound P (Ponnamperuma, 1972; Baldwin and Mitchell, 2000; Liptzin and Silver, 2009). This P can be subsequently resorbed or taken up by biota, and the sorption–desorption cycle of Fe and P bonding (in part determined by the soil and climate of tropical forests) helps regulate P cycling and availability.

Soil organic matter (SOM) is an important influential factor for chemical, physical and biological soil properties. Aside from iron oxides, SOM is the soil constituent that most strongly affects the reactions and rate of P adsorption and desorption, especially in highly weathered soils (Fink et al., 2016). SOM can supply P to plants by either blocking absorption sites and/or releasing soluble P with time. Negatively charged functional groups in organic substances (e.g., carboxyl, phenol) can interact with positively charged minerals, such as iron oxides, altering P adsorption as a result (Schwertmann et al., 1986; Liu et al., 1999). Adsorption of organic functional groups onto iron



oxides can promote anion adsorption via cation bridges ( $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$ ), boost competition with other anions for adsorption sites and cause adsorbed anions, such as P, to be desorbed (Hinsiger et al., 2011; Borggaard et al., 2005; Guppy et al., 2005). In addition, SOM is a primary energy resource for organisms involved in nutrient recycling and soil structuration. SOM is used as a source of carbon (C) and energy by microorganisms to fuel and stimulate the microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  minerals, thereby releasing substantial amount of P associated with  $\text{Fe}^{3+}$  oxides (Rakotoson et al., 2015; Scalenghe et al., 2002). With regard to soil physical properties, SOM has a positive effect on soil aggregation, which enhances water infiltration. Enhanced water infiltration reduces erosion (Bronick & Lal, 2005), keeping P available for biological uptake.

### *1.1.4.2 Biotic control: Vegetation, Soil Fauna*

Plants take up or mine easily-available P in the soil, which is considered as one source of P output from the soil. However, litterfall or other organic materials from plants contribute to the easily-available  $\text{Po}$  pool, which acts as a P reserve for buffering available P following decomposition and mineralization in P-depleted soil (Maranguit et al., 2017). Plants form a symbiotic relationship in their rootlets with microorganisms like mycorrhizal fungi, excreting phosphatase and organic acids to release P and providing an active uptake site for the rapid diffusion of P from soil pore spaces to the root surface (Antibus et al., 1981, Bolan et al., 1984, Dodd et al., 1987). In exchange, the plant provides carbohydrates to the mycorrhizal fungi (Schlesinger, 1997).

Soil organisms are the driving force behind plant nutrient transformation and play a crucial role in soil fertility and ecosystem functioning (Smith and Paul 1990; Spohn and Kuzyakov, 2013; Damon et al., 2014). In P cycling, microorganisms processes, which include, (1) microbial P immobilization, (2) re-mineralization of immobilized P and (3) mineralization of non-microbial organic P (SOM), are of great importance, especially in soil with low P availability. Soil microbes secrete phosphatase, an enzyme that can release bio-available inorganic P from organic matter (Kroehler and Linkins, 1988, Tarafdar and Claasen, 1988; Nannipieri et al., 2011; Rasavi et al., 2016).

Without phosphatase enzymes, the presence of Pi would be limited to external sources, such as fertilizers, and primary productivity would be limited and dependent on these external sources.

Aside from being the main mediator of SOM mineralization (and hence, increasing P availability), microbial uptake of P acts as a very important labile reservoir, providing significant contributions to plant available P pools following microbial biomass turnover (Brookes et al., 1984; Frossard et al., 2011; Bünemann, 2015; Yevdokimov et al., 2016). The P content stored in the microbial biomass constitutes a significant component of total soil P, and is generally larger than the P content in above-ground biomass (Perrott and Sarathchandra, 1989, Richardson and Simpson, 2011). Thus, microorganisms effectively compete with plants for available orthophosphate from soil solution and also represent a significant pool of immobilized P that is temporarily unavailable to plants (Richardson and Simpson, 2011). Nonetheless, immobilization of P within the biomass was suggested to be an important mechanisms for regulating the supply of P in soil solution (Seeling and Zasoski, 1993), protecting P from erosion or leaching and from fixation to oxides, thereby maintaining P in labile forms (Olander and Vitousek, 2004). Over the longer term, the rapid turnover of microbial biomass P makes this pool a relevant dynamic source of plant-available P (Bünemann, 2015).

Earthworms, a major component of soil faunal communities in most ecosystems, also play a crucial role in P cycling. Their activity is beneficial because it can enhance soil nutrient cycling and soil aggregation through the rapid incorporation of detritus into mineral soils (Bhadoria and Saxena, 2010). Earthworm burrowing activities produces pores (i.e., Biopores) in the soil and/or on the soil surface (Hoang et al., 2016) where beneficial soil microorganisms can colonize. As earthworms dig burrows they deposit casts, which is digested organic material formed while mixing soil horizons and burying above ground litter. The casts and mucus production associated with water excretion from earthworm guts also enhance the activity of beneficial soil microorganisms colonizing the biopores (Bhadoria and Saxena, 2010). For example, when bacteria colonize biopores, the activity of phosphatase, an enzyme responsible for solubilizing P, increases in the soil (Wan et al., 2004).

### *1.1.4.3 Anthropogenic control: Land-use change*

P is a key nutrient requiring attention in response to human activities (Garcia-Montiel et al., 2000). Human impact on the P cycle has been substantial over the last 150 years and will continue to dominate the natural cycle of P in the future (Filippelli, 2002). Land use change via fire, forest change to pasture, deforestation, agriculture, and urbanization will certainly alter P cycling, especially in tropical ecosystems, and multiple lines of evidence suggest that such perturbations may enhance P losses and exacerbate P limitation (Maranguit et al., 2017). This will result in potentially significant effects on the distribution of P within chemically-defined pools, in turn determining availability and stability (Wright, 2009). It is thought that the biggest changes affecting the availability of P over time are result from inorganic and organic fertilizer application (Neufeldt et al., 2000; Guo et al., 2000). The use of fertilizers has been mentioned as the most important method of increasing inorganic and easily available soil P forms. It accomplishes the task of maintaining enough available P for crops (Sample et al., 1980; Beauchemin and Simard, 2000). Nonetheless, P fertilization is not sustainable in the long-run, as rock phosphate reserves suitable for fertilizer production are rapidly declining, rendering P fertilizer increasingly expensive (Cordell et al., 2009).

Moreover, the exhaustion of SOM resulting from the conversion of natural forest to plantations raises major concerns for its functions (e.g., P fertility, soil compaction – erosion/flooding) directly or indirectly affecting soil P availability.

### *1.1.5 Challenges for P availability*

In order to enhance P availability in P-depleted highly weathered soil and increase the efficiency of P fertilizers, management practices must be enhanced. A better understanding of P dynamics and the mechanisms controlling availability are the initial steps in improving management practices. Furthermore, quantifying P losses due to land-use changes and their effects on soil functions affecting P availability must be determined to achieve optimum land productivity.

## 1.2 Objectives

The main objectives of the present work were as follows:

- 1.2.1 Evaluation of methodology to determine applicability for the purpose of P determination in various P pools of different availability (study 1)
  - $^{33}\text{P}$  isotope-labeling technique for tracing the fates of P fertilizer applied to soils
  - Hedley et al (1982) sequential P fractionation method for extracting P from various pools
  
- 1.2.2 Elucidate the role of abiotic and biotic processes in conserving P availability in P-limited soils.
  - determine the rate of incorporation of newly added P fertilizer into the various P pools of different availability (study 1)
  - demonstrate the effect of labile organic carbon on microbial activity controlling P availability (studies 1, 3, 5)
  - show the contribution of macro fauna (earthworms) to P mobilization and availability (study 6)
  - determine the effect of P adsorption by Fe and Al oxides on P availability (studies 1, 2, 3)
  - investigate the effect of land-use type on phosphatase enzyme activity, which is responsible for solubilizing P (studies 4, 5)
  
- 1.2.3 Impact assessment of land-use change on P availability
  - quantify P losses following forest change to oil palm and rubber tree plantations (studies 2, 4)
  - identify the mechanisms controlling P availability and losses under different land-use types (studies 2, 4, 7)
  - assess the effect of SOM exhaustion on P availability following forest change to agricultural land-use (studies 2, 3, 7)
  - assess the effect of soil flooding resulting from soil compaction after forest change to monoculture plantation on P mobilization (study 3)

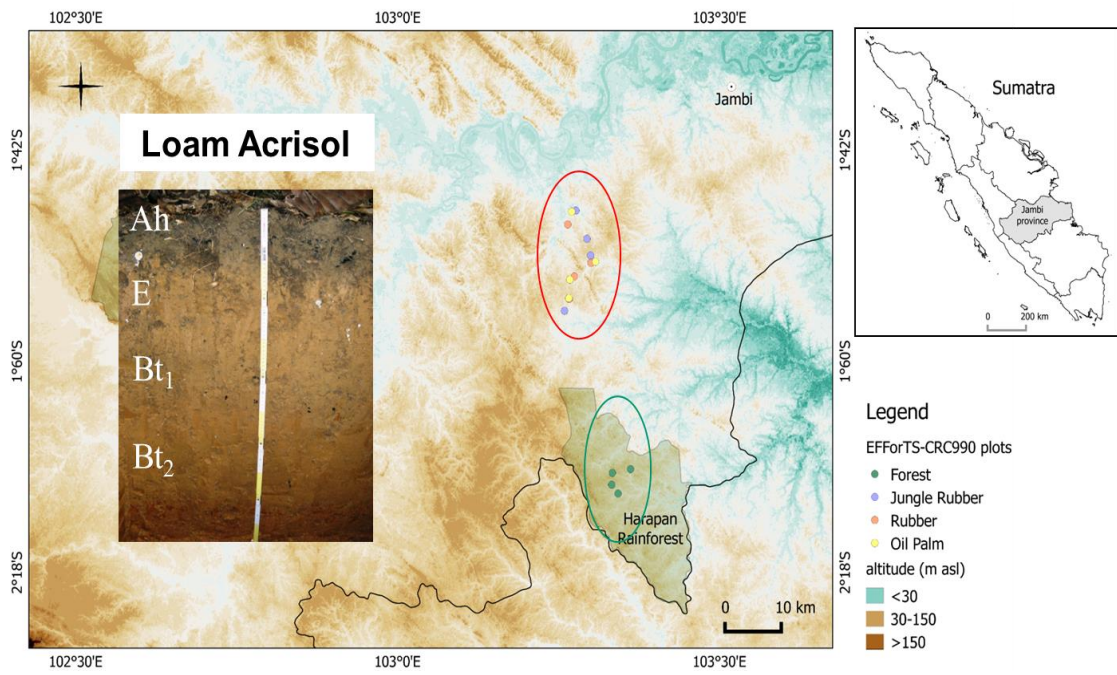
### 1.3 Materials and Methods

#### 1.3.1 Study area and soil sampling

For the study focused on identifying the role of biotic and abiotic factors in altering P dynamics and assessing the efficiency of the P fractionation method (study 1), bulk soil from the Ah horizon at site Unterluess (Luess) was used. The site was located in the district Celle of Lower Saxony (Germany) in the Lueneburg Heath (52°50.32 'N, 10°16.0 'E) at 115 m a.s.l. The soil type is a Hyperdystric Folic Cambisol developed from Pleistocene sediments.

Tropical and sub-tropical soils were considered in the studies investigating the impact of land-use change on P dynamics and its effect on biotic and abiotic processes of P availability. The study in the tropics was carried out in the Jambi Province in Sumatra, Indonesia (Fig. S3). The climate is tropical humid with an average temperature of 27 °C and an average precipitation of 2200 mm yr<sup>-1</sup> and 112–259 mm month<sup>-1</sup> (Guillaume et al., 2015). Aside from tropical rainforest, the area had three dominating land-use types (Fig. S4): (1) extensively-managed agroforest (jungle rubber) in which rubber trees are planted in a partially logged forest, (2) intensively-managed rubber plantation, and (3) oil palm plantation. Three replicate sites for each land-use type were selected within a distance of 16 km with an elevation varying between 50 and 100 m a.s.l. The soils were Acrisols with a sandy loam texture.

The study in sub-tropics was carried out in the Chitwan district (27° 35'N 84° 30'E) of Nepal with an annual rainfall of 1763 mm and an average temperature of 30°C. Three land-use types were considered: forest, organic and conventional farming. Samples were collected in topsoil (0–10 cm) and subsoil (10–20 cm).



**Figure S3** Study site, sampling area and the typical soil profile of a loam acrisol that can be observed at Sumatra Indonesia.



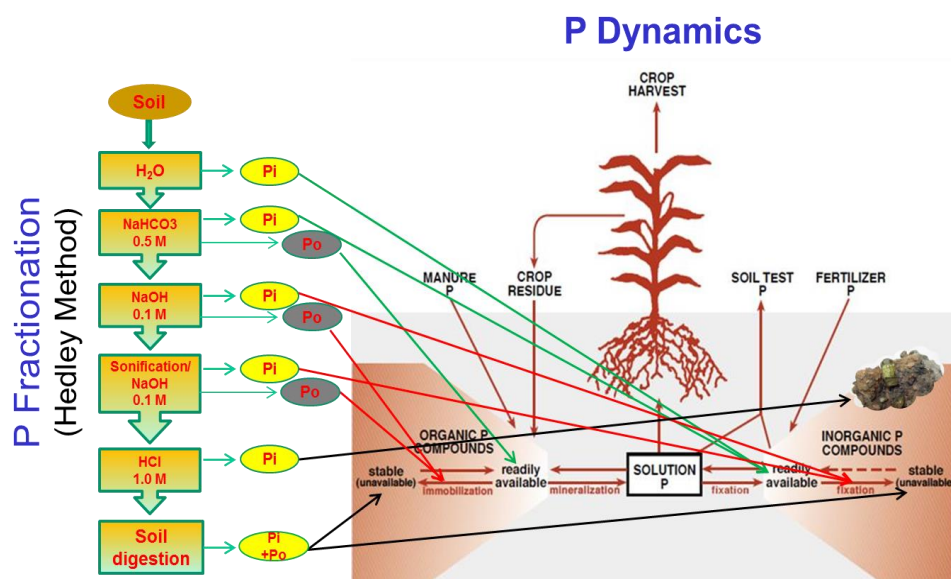
**Figure S4** Three dominating land-use types in Sumatra Indonesia after forest conversion.

### 1.3.2 $^{33}\text{P}$ isotope-labeling technique

The  $^{33}\text{P}$  isotope-labeling technique was used in an incubation experiment (study 1) to compare the fate of P applied at increasing rates to acidic, P-limited soil depending on the presence of C and N sources applied as glucose and ammonium sulfate, respectively. The incorporation of  $^{33}\text{P}$  from  $\text{KH}_2\text{PO}_4$  into various P pools (i.e. microbial P ( $\text{P}_{\text{mic}}$ ), available P ( $\text{P}_{\text{AEM}}$ ) and Fe-bound P ( $\text{P}_{\text{NaOH}}$ )) was traced.

### 1.3.3 Hedley et al. (1982) sequential P fractionation method

The Hedley et al. (1982) sequential fractionation method was used to fractionate soil P in all of the studies. Hedley fractionation assumes that extractants of varying strength estimate  $\text{P}_i$  and  $\text{P}_o$  fractions of different availability and chemical bindings (Guo et al., 2000; Hedley et al., 1982) (Fig. S5).



**Figure S5** The Hedley et al. (1982) sequential P fractionation method.

### 1.3.4 Flooding simulation experiment

2.5 g of soil sample were filled into a 12 ml glass tube (Labco Exetainer). Six milliliters of purified distilled water were added in each tube and air was driven out by purging with  $\text{N}_2$  gas. The suspension was then covered with a rubber stopper to prevent  $\text{O}_2$  diffusion, evaporation losses and to ensure anaerobic conditions. Four field replicates of each land-use type and depth were incubated in the dark at  $30 \pm 1$  °C.

## 1.4 Main results and discussion

### 1.4.1 Method Applicability

- Through the use of the  $^{33}\text{P}$  isotope-labeling technique, the Hedley et al. (1982) sequential fractionation method was found to be very efficient and accurate in extracting P from various P pools. Recovery of around 86% of labelled  $^{33}\text{P}$  in extracted P pools (i.e., available and Fe-P pools), suggests that Hedley fractionation is a good method to use in assessing P distribution in soils under different land-use managements. It also provides an estimate of the P forms that may potentially contribute to available P over a growing season.
- The use of  $^{33}\text{P}$  as a tracer in the incubation experiment (study 1) provides information on the contribution of abiotic and biotic factors to P availability. In addition, the use of the  $^{33}\text{P}$  isotope-labelling technique provides information on the gross P fluxes in the respective P pools (Fig. S6).

### 1.4.2 Role of abiotic and biotic processes on P availability

- Abiotic and biotic factors were found to influence the forms and distribution of P in various P pools of different availability (Table S1). These either increased or decreased P content in different pools.

**Table S1** Direct effects of abiotic and biotic processes on P fractions in soil.

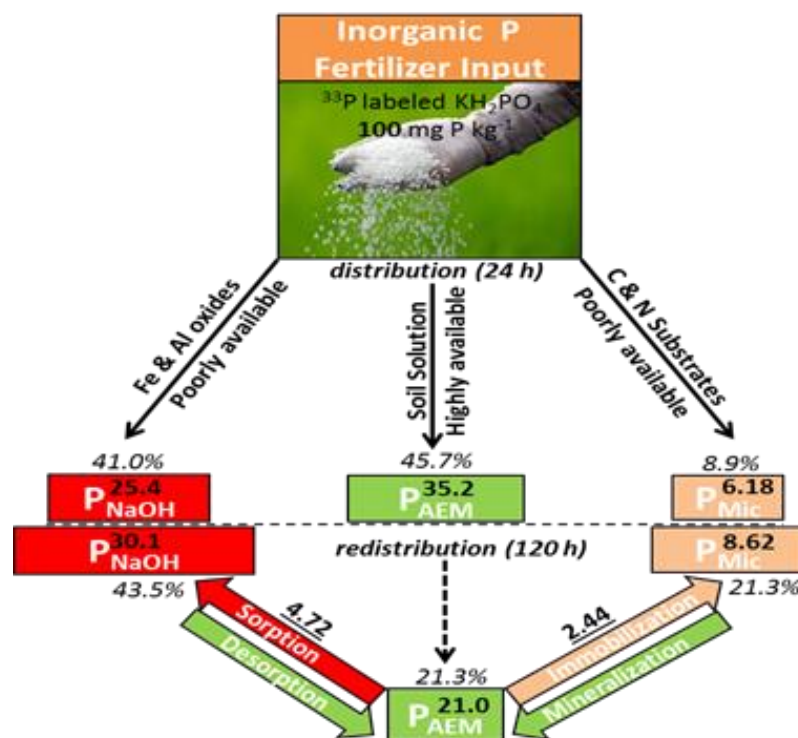
Processes/Mechanism		Easily-available		Moderately-available		Non-available
		Pi	Po	Pi	Po	Pi + Po
Biotic	Litter input	↑	↑↑		↑	
	Mineralization	↑↑	↓↓		↓	
	Plant uptake	↓↓↓	↓↓	↓↓	↓	
	Immobilization in microorganism	↓↓↓	↓↓		↑↑	↑↑
Abiotic	Erosion	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
	Leaching	↓↓	↓	↓		
	Fixation/Adsorption	↓↓↓	↓↓	↑↑	↑	↑↑
	Desorption	↑↑		↓↓		↓

↑↑↑ strong increase; ↑↑ moderately increase; ↑ slight increase

↓↓↓ strong decrease; ↓↓ strong decrease; ↓ slight decrease



- Indeed, abiotic and biotic factors control P availability in the soil as observed from our experiment (study 1). After the addition of  $^{33}\text{P}$ -labelled fertilizer, a fast, almost instantaneous P fixation by the Fe and Al oxides and immobilization by microbial uptake were observed (Fig S6).
- Applying glucose boosts microbial growth and demand for P, resulting in increased  $^{33}\text{P}$  recovery (20% of the applied  $^{33}\text{P}$ ) and P content in  $\text{P}_{\text{mic}}$ .
- The negative relationship between  $\text{P}_{\text{mic}}$  and  $\text{P}_{\text{AEM}}$  ( $P < 0.05$ ;  $R^2 = 0.46$ ) emphasizes that P availability is influenced by microbial uptake. The high  $^{33}\text{P}$  recovery (45% of applied  $^{33}\text{P}$ ) in  $\text{P}_{\text{NaOH}}$  and the strong negative relationship ( $P < 0.001$ ;  $R^2 = 90-96$ ) between  $\text{P}_{\text{NaOH}}$  and  $\text{P}_{\text{AEM}}$  demonstrate the dominance of P adsorption by Fe and Al oxides as a potential P fate.
- Therefore, P availability is strongly mediated both by physicochemical and biological reactions. These two process groups – biotic (microbial P immobilization) and abiotic (adsorption) – sustain long-term P fertility *via* the turnover of microbial biomass and desorption of fixed P, respectively.

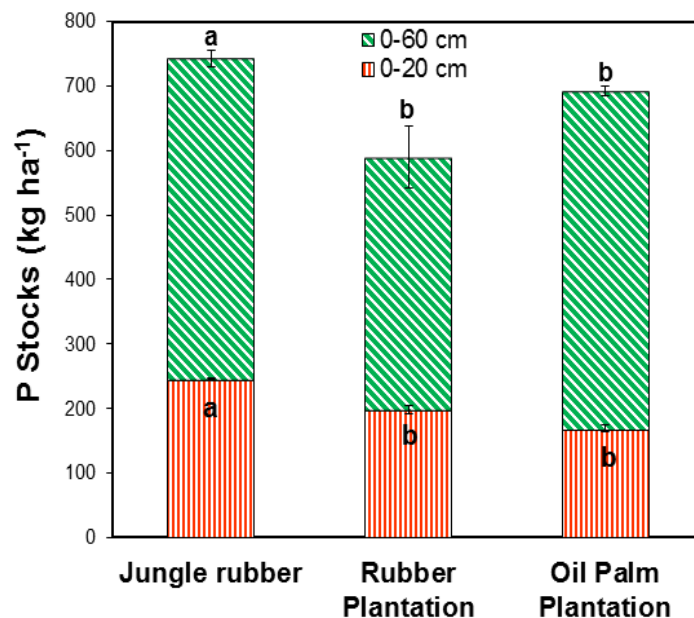


**Figure S6** The fate of inorganic phosphorus applied to acidic P-limited soil. The distribution of P fertilizer in P pools is affected by biological and physicochemical reactions. Box size indicates the proportion of P fertilizer recovered in each pool throughout the incubation period. Values in bold inside the box: P content in respective pool (mg P kg<sup>-1</sup>); values in italics:  $^{33}\text{P}$  recovery in respective pool (% of applied  $^{33}\text{P}$ ); underlined values: the total increase of P content (mg P kg<sup>-1</sup>) immobilized and adsorbed at 120 h. Values above and below horizontal dashed line represent contents at 24 h and 120 h, respectively.

- Earthworms influence P availability. The biopores created by earthworms were considered hotspots for microbial activity, which contributes to P mobilization. This was shown by higher MBP content and phosphatase enzyme activity in biopore soils (both topsoil and subsoil) compared to both earthworm-free (control) and undigested soils.

#### 1.4.3 Land-use control on P availability

- Land-use change leads to an overall reduction in P stocks (Fig. S7), resulting from a strong decrease in SOM content caused by erosion and yield export of rubber or oil palm seeds.

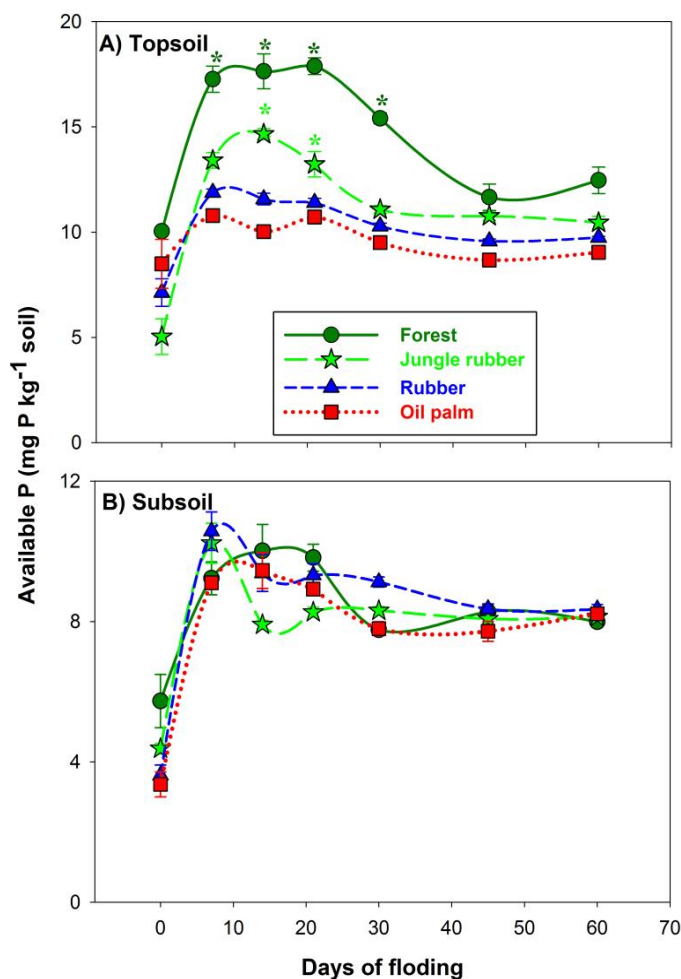


**Figure S7**

Soil phosphorus stocks (kg P ha<sup>-1</sup>) at the 0-20 and 0-60 cm soil depth layer depending on land use. Values represent means  $\pm$  SE (n=3). Means followed by different letters within the same depth differ significantly (*t*-test at  $P < 0.05$ ).

- Fertilization did not compensate for these additional P losses. Fertilization only increases the available Pi in the topsoil. While this maintains or increases fertility over the short term, it ultimately results in decreases fertility over the long term by depleting P reserves (e.g., moderate or non-available P).
- Acid phosphatase activity, responsible for the hydrolysis of recalcitrant organic P, was also altered following land-use change. Acid phosphatase activity was lower in monoculture plantation soils (48–71 nMg<sup>-1</sup> h<sup>-1</sup>) than in forest (189 nMg<sup>-1</sup> h<sup>-1</sup>) and agroforest soils (93 nMg<sup>-1</sup> h<sup>-1</sup>).

- Land-use type influenced the impacts of flooding on P and Fe forms mainly in the topsoil, where P dissolution and availability were generally higher under forest and, to a lesser extent, under jungle rubber. Faster solubility in topsoil and especially under forest is connected with a higher SOM content, which influences microorganisms (Fig. S8).
- SOM is used as a source of C and energy by the microorganisms and stimulates the microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  minerals. The presence of high concentrations of native SOM in forest and jungle topsoils drove  $\text{Fe}^{3+}$  reductions and increased the reduction intensity.
- In addition to serving as a C source for microorganisms, SOM was found to contribute to the available P content. This was demonstrated by the strong positive correlation between soil C and  $\text{P}_o$  and the C: $\text{P}_o$  ratio in agroforest soils.



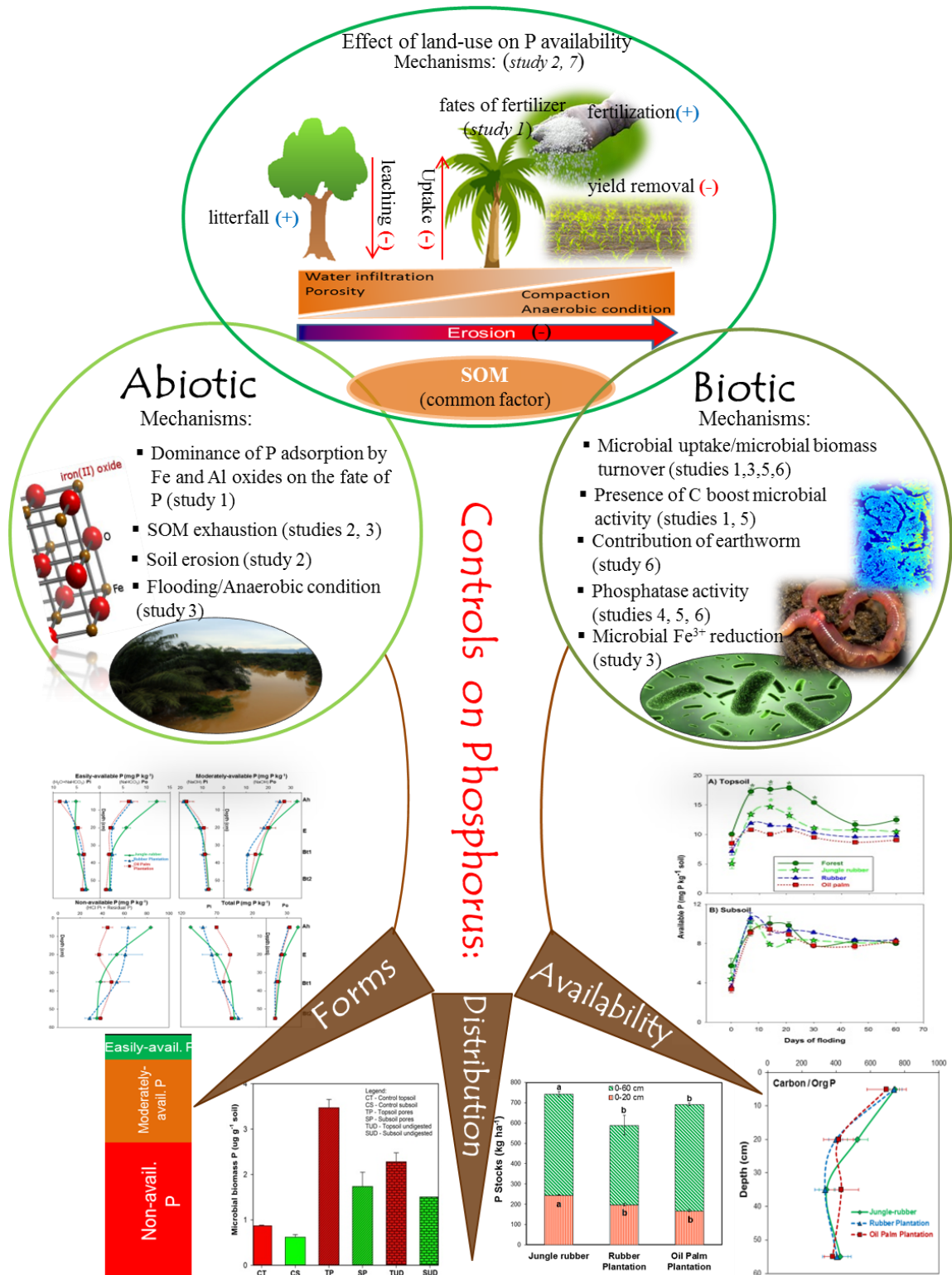
**Figure S8** Effects of soil flooding on the available P ( $\text{P}_{\text{NaHCO}_3}$ ) of topsoil (A) and subsoil (B) under forest, jungle rubber, rubber and oil palm plantations. Available P on day 0 indicates the initial content before soil flooding. Values represent means  $\pm$  SE ( $n=4$ ). Asterisks show significant differences (one-way ANOVA;  $p < 0.05$ ) to rubber and oil palm soils at a given sampling time.

- Under natural condition, however, we speculate that the P turnover is faster in compacted soils under plantations due to the regular changes between oxic and anoxic conditions. Consequently, more P is taken up by the vegetation and subsequently removed from plantations *via* yield export.

### 1.5 Conclusions

The form, distribution and availability of P in P-limited soils are determined by abiotic and biotic processes. When P fertilizer is introduced to the system, it will be quickly immobilized by microorganisms and fixed by Fe and Al oxides. In highly weathered soil (e.g. most tropical soils) with high concentration of Fe and Al oxides, fixation of P dominated P availability. On the other hand, if soil contains high amount of SOM, an important source of energy for microorganisms, the growth of microorganisms will increase and the demand for P will also increase. Microorganisms will take up high levels of P, which will be incorporated into their biomass. All of these scenarios were proven and traced *via* the application of a novel  $^{33}\text{P}$  isotope-labeling technique in an incubation experiment, followed by careful extraction of the various P pools using the Hedley et al. (1982) sequential P extraction method. Furthermore, our results suggest that turnover of microbial biomass and desorption of P sustains the long-term P fertility by minimizing P losses from erosion and/or leaching. Earthworms also contribute to P mobilization, as demonstrated by the high MBP content and phosphatase enzyme activity inside earthworm biopores. Nonetheless, anthropogenic activities, such as the conversion of forest to monoculture plantations or for any other agricultural use, were found to alter abiotic and biotic mechanisms of P mobilization, especially in P-limited soils. Although plantation with high levels of P fertility exhibit normal yields, fertilization only maintains short-term fertility but is not sustainable in the long-run due to the depletion of P reserve pools (i.e., organic P, moderately and non-available P). Acid phosphatase activity, responsible for the hydrolysis of recalcitrant organic P to make it available for plant uptake, was also affected by land-use change. The exhaustion of SOM mainly affects P mobilization following change of forest to monoculture plantation. The SOM itself

contributes to the available P pool once decomposed and mineralized. However, in plantation systems, plant biomass (i.e., yield of rubber and/or oil palm seed) is exported, reducing easily mineralizable  $P_o$ . The depletion of SOM also affects soil physical properties, such as bulk density, which have a direct effect on P availability. Compacted soils are prone to erosion, which is one of the outputs of P losses. Nonetheless, the vulnerability of plantations to erosion or flooding can enhance mobilization of fixed P through microbial activity. Microbially-mediated  $Fe^{3+}$  reduction and the associated P release during anaerobic conditions play a fundamental role in plant and microorganism nutrient status by releasing inaccessible P during dry conditions. However, this microbially-mediated reductive dissolution of ferric oxides was apparently determined by the SOM content, as it is a source of the C and energy required for microorganisms to efficiently reduce  $Fe^{3+}$ . Therefore, management practices should be improved to provide higher biomass SOM input while mitigating soil erosion in order to attenuate P depletion.



**Figure S9** Synthesis of the main results. Positive (+) and negative (-) signs indicate increases and decreases, respectively, in P availability and content.

## 1.6 References

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## 2 Publications and Manuscripts

### 2.1 Study 1: Biotic and abiotic processes of phosphorus cycling in acidic P-limited soil: $^{33}\text{P}$ tracer assessment

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#### 2.1.1 Abstract

$^{33}\text{P}$  labeling has high sensitivity to trace soil P dynamics and the fate of added P fertilizers across various P pools. Nonetheless, only a few studies used this approach. This study was designed to investigate the P dynamics and to assess the effects of biological and physicochemical processes on P availability in acidic P-limited soil. We followed the incorporation of  $^{33}\text{P}$ -labeled  $\text{KH}_2\text{PO}_4$  in available P ( $P_{\text{AEM}}$ ), microbial biomass P ( $P_{\text{mic}}$ ) and Fe/Al-bound P ( $P_{\text{NaOH}}$ ) pools in Cambisol – depending on the presence of carbon and nitrogen sources applied as glucose and ammonium sulfate, respectively. Not all applied P fertilizer is available for plant uptake; instead, it was distributed to poorly-available pools. We recorded fast, almost instantaneous P fixation by the Fe and Al oxides and immobilization by microbial uptake. Applying glucose boosts microbial growth and demand for P, resulting in increased  $^{33}\text{P}$  recovery (20% of the applied  $^{33}\text{P}$ ) and P content in  $P_{\text{mic}}$ . The negative relationship between  $P_{\text{mic}}$  and  $P_{\text{AEM}}$  ( $P < 0.05$ ;  $R^2 = 0.46$ ) emphasizes that P availability is influenced by microbial uptake. The high  $^{33}\text{P}$  recovery (45% of applied  $^{33}\text{P}$ ) in  $P_{\text{NaOH}}$  and the strong negative relationship ( $P < 0.001$ ;  $R^2 = 90-96$ ) between  $P_{\text{NaOH}}$  and  $P_{\text{AEM}}$  show the dominance of P adsorption by Fe and Al oxides on the fate of P. Therefore, P availability are strongly controlled both by physicochemical and biological reactions. These two process groups – biotic (microbial P immobilization) and abiotic (adsorption) – sustain long-term P fertility after the turnover of microbial biomass and desorption of fixed P, respectively.

**Keywords:**  $^{33}\text{P}$  isotopic labeling; Phosphorus dynamics; Phosphorus fractions and availability; Microbial biomass P; Sorption-desorption; Cambisol

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### 2.1.2 Introduction

The limited availability of phosphorus (P) is often the main constraint for plant growth and productivity, especially in acidic soils (Bünemann et al. 2004; Turner et al. 2012, 2013; Maranguit et al. 2017). This P limitation for crop production puts considerable pressure on farmers to supply the soil with P fertilizer in order to meet demand. However, the decreasing rock phosphate resources suitable for fertilizer production (Cordell et al. 2009) make P fertilizer increasingly expensive. This calls for a better understanding of P dynamics in soil. The aim is to plan more effective soil management practices to increase the efficiency of P fertilizers (Aulakh et al. 2003) and achieve optimum plant growth (Damon et al. 2014).

The fate of P in soils is mainly governed by anthropogenic, biotic and abiotic processes that either increase or decrease the soil P content and availability (Frossard et al. 2000; Nannipieri et al. 2002; Maranguit et al. 2017). Biotic processes such as microbial immobilization, re-mineralization of immobilized P and mineralization of non-microbial organic P by microorganisms are crucial for P cycling in soil (Nannipieri et al. 1978, 2011; Frossard et al. 2000, 2011; Bünemann 2015; Yevdokimov et al. 2016). Although microbial biomass P ( $P_{\text{mic}}$ ) is one of the insoluble P forms in soil, it is a potentially available P source for plant uptake (Blackwell et al. 2010; Spohn and Kuzyakov 2013; Damon et al. 2014; Yevdokimov et al. 2016). This pool plays a key role in the P dynamics in soils by immobilizing inorganic P, which may then be released slowly and taken up by crops more efficiently during microbial biomass turnover (Brookes et al. 1984; Joergensen et al. 1995) or upon microorganism death. P availability also depends partly on abiotic processes that mainly reduce P availability (Frossard et al. 2000). These include phosphate fixation to the solid phase, e.g., formation and precipitation of  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  phosphates, binding phosphates in complexes with SOM, sorption on clay minerals, etc. (Frossard et al. 2000).

The “Isotopic Labeling Technique” has the potential and very high sensitivity to elucidate soil P dynamics and the fate of P across the various P pools. This technique consists of two approaches: labeling and tracing the fate of a P source, and isotopic dilution. By applying the  $^{33}\text{P}$  as a tracer in incubation experiments, the contribution of physicochemical processes delivering available P into the soil solution can be differentiated from biological P processes and the respective gross P fluxes (Oehl et al. 2001; Bünemann et al. 2004, 2007; Dijkstra et al. 2015). Nonetheless, only a few studies have used this approach to assess the fate of P, its transformation and the rate of the reactions as influenced by biological and physicochemical processes in acidic P-limited soil. The objective of this study was to trace the fate of P and investigate if biological and physicochemical processes determine P availability in acidic P-limited soil.

We used the isotopic labeling technique in an incubation experiment to compare the fate of P applied at increasing rates to acidic P-limited soil – depending on the presence of C and N sources applied as glucose and ammonium sulfate, respectively. We followed the incorporation of  $^{33}\text{P}$  from  $\text{KH}_2\text{PO}_4$  as a P source into various P pools (i.e. microbial P ( $P_{\text{mic}}$ ), available P ( $P_{\text{AEM}}$ ) and Fe-bound P ( $P_{\text{NaOH}}$ )). Application of solely  $^{33}\text{P}$  tracer added in distilled water was used as a control to determine soil P dynamics in the absence of P additions. We hypothesized that: (1) P availability is greatly influenced by microbial activity and by P adsorption by Fe and Al oxides; (2) available P will be (re)distributed quickly to P pools with less availability; (3) carbon and nitrogen addition will boost microbial growth and activity, further affecting microbial P uptake and, thus, the P dynamics.

### **2.1.3 Materials and Methods**

#### *2.1.3.1 Site and soil sampling*

Soil samples were taken at site Unterluess (Luess) located in the district Celle of Lower Saxony (Germany) in the Lueneburg Heath (52°50.32 'N, 10°16.0

**Table 1** General characteristics of the Cambisol used in the experiment (Bergkemper et al. 2016)

Soil	Geographic Location	Soil Type (WRB, 2015)	Depth (cm)	Total C (mg g <sup>-1</sup> )	Total N (mg g <sup>-1</sup> )	Total P (µg g <sup>-1</sup> )	pH (H <sub>2</sub> O)	Sand (%)	Silt (%)	Clay (%)	Texture (WRB, 2015)
Luess (LUE)	52°50'21.77" N 10°16'2.37" E	Hyperdystric Folic Cambisol	0-20	43.0	1.70	144	3.72	77.7	17.3	5.33	Loamy sand

'E) at 115 m a.s.l. The mean annual rainfall is 780 mm, the mean annual temperature 8.0 °C. The soil type is a Hyperdystric Folic Cambisol developed from Pleistocene sediments. The vegetation is formed by European beech (*Fagus sylvatica* L.). The total P in the soil is very low (Table 1; Bergkemper et al. 2016). This site therefore well represents acidic soils with very low P availability. A bulk sample of the Ah horizon at 0-10 cm depth was collected with a shovel after removal of the organic layer. The sample was sieved (2 mm) and stored at +4 °C.

#### 2.1.3.2 Experimental design

The fate of P fertilization combined with C and N in Cambisol was studied in laboratory incubation. The experiment was full factorial, composed of 12 experimental units. The main factor was the rate of P addition, which includes 0%, 10% and 50% of the initial total P content. Each P level was amended with the following (second factor): (1) glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>) as a C source in a one-time and staggered application, (2) ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] as an N source and (3) distilled water as a control. Each treatment was

replicated four times. Fifteen grams of dry weight equivalent soil (sieved at 2 mm) were placed into glass jars with caps and pre-incubated at 25 °C and 50% water holding capacity (WHC) in the dark to stabilize microbial activity

until a constant CO<sub>2</sub> rate was reached on three consecutive days. After pre-incubation P, C, N and distilled water were added.

Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) labeled with <sup>33</sup>P was used as P fertilizer. Three P levels were added: i) no P = 0.3 mL deionized water + <sup>33</sup>P tracer only (P<sub>0</sub>); ii) 10% P from initial extractable soil P (0.2 mg g<sup>-1</sup>) = 0.3 mL solution of 4.4 mg KH<sub>2</sub>PO<sub>4</sub> dissolved in 1 mL of deionized water (P<sub>10</sub>); iii) 50% P from initial extractable soil P = 0.3 mL solution of 21.95 mg KH<sub>2</sub>PO<sub>4</sub> dissolved in 1 mL of deionized water (P<sub>50</sub>). An addition of 10% P and 50% P increases initial extractable P by 20 µg g<sup>-1</sup> and 100 µg g<sup>-1</sup>, respectively. <sup>33</sup>P labelling resulted in an addition of 80 Bq per gram soil.

Each level of P received: i) deionized water (control); ii) 50 µg C g<sup>-1</sup> soil in a one-time application = 0.3 mL solution of 6.25 mg glucose dissolved in 1 mL of deionized water (C<sub>50</sub>); iii) 5 times each day in the total amount of 10 µg C g<sup>-1</sup> soil day<sup>-1</sup> = 0.3 mL solution of 1.25 mg glucose dissolved in 1 mL of deionized water (C<sub>10x5</sub>); iv) 50 µg N g<sup>-1</sup> soil = 0.3 ml solution of 3.53 mg (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> dissolved in 1 ml of deionized water (N<sub>50</sub>).

To maintain constant WHC at 10 µg C g<sup>-1</sup> soil day<sup>-1</sup> (C<sub>10x5</sub>) treatment, plastic caps with Silica gel with indicator (Merck Millipore, Germany) were placed into each jar and replaced every day after addition of glucose solution. The amount of silica gel needed to decrease WHC by 20% in 15 g soil was determined in a preliminary experiment. Incubation lasted for 6 days at 70% WHC and 25 °C in the dark. Two samplings were done: after 24 h and 120 h of incubation.

### 2.1.3.3 Phosphorus fractionation

Microbial P was determined by simultaneous liquid chloroform-fumigation and extraction with anion exchange resin membranes (AEM) (BDH no. 551642S, 1.5 x 6.25 cm – with a reactive area of 18.75 cm<sup>2</sup> per strip) in bicarbonate form (Kouno et al. 1995). Briefly, 3 g of subsamples were put in a 50 ml centrifuge tube and mixed with 0.3 ml chloroform and 30 ml deionized water (fumigated soil). Another 3 g of subsamples were mixed with only 30 ml

deionized water as a control (unfumigated soil). AEMs were added to both samples (fumigated and unfumigated) and tubes were shaken for 24 h in an orbital shaker. After exposure to test solution, the membranes were removed with tweezers, washed 4 times in deionized water and shaken dry to remove excess water. Thereafter, P from the membranes was desorbed with 45 mL of 0.25 M H<sub>2</sub>SO<sub>4</sub> by shaking for 3 h. Phosphate and <sup>33</sup>P activity in the extract were measured (see section 2.4 and 2.5).

The soil samples (fumigated sample) remaining after fumigation-extraction were further extracted using 30 ml of 0.1 M NaOH to extract the P pool that was adsorbed by Fe/Al oxides (Hedley et al. 1982; Maranguit et al. 2017). Samples were shaken for 24 h in an orbital shaker, centrifuged at 5000 rpm for 15 min and filtered using Whatman no. 42 filters. Extracts for P measurement were acidified using 0.9 M H<sub>2</sub>SO<sub>4</sub> to precipitate dissolved organic matter that could interfere in color development and in the measurement. Phosphate and <sup>33</sup>P activity were also measured in the extract (see section 2.4 and 2.5).

### 2.1.3.4 Phosphate measurement

Phosphate in the fumigated, unfumigated and NaOH-extracts was determined by the malachite green (MG) colorimetric method (D'Angelo et al. 2001). Briefly, 150 µl of extracts was mixed with 30 µl of the first reagent (ammonium molybdate tetrahydrate and sulfuric acid) in a disposable 96-well polystyrene microtiter plate. It was shaken for 10 min in an orbital shaker at low speed (<90 rev min<sup>-1</sup>). Thereafter, we added 30 µl of the second reagent, which was a mixture of MG carbinol hydrochloride and polyvinyl alcohol. The plate was shaken for an additional 20 min. Thereafter, samples were exposed to 40 °C for 40 min. Absorbance was read after 1-1.5 h using a spectrophotometer (TECAN; Infinite M200 pro) with 630 nm wavelength. To decrease the variability of the replicates, microplates were left overnight and read again for absorbance. Standards were also prepared in triplicate and treated the same way as the samples. P<sub>mic</sub> (mg P kg<sup>-1</sup>) was calculated as:

$$P_{mic} = (P_{fumigated} - P_{unfumigated}) / K_p, \quad [1]$$

where  $P_{\text{fumigated}} = P_{\text{AEM}}$  in fumigated samples in  $\text{mg P kg}^{-1}$ ,  $P_{\text{unfumigated}} = P_{\text{AEM}}$  in unfumigated samples in  $\text{mg P kg}^{-1}$  and  $K_p$  is the correction factor to account for the effect of sorption and isotopic exchange and extraction efficiency.  $R_{\text{sorp}}$  and  $R_{\text{exch}}$  are equal to 0.9 (Bergkemper et al. 2016; Yevdokimov et al. 2016). The soil specific correction factor ( $K_p = 0.69$ ) was determined for the soil used in this experiment.

### 2.1.3.5 $^{33}\text{P}$ activity measurement and calculations

One ml of the fumigated, unfumigated and NaOH-extract were transferred into 6 ml vials and mixed with 3 ml of scintillation cocktail Rotiszint EcoPlus (Carl Roth Company, Germany) and were measured using a HIDEX 300 SL Liquid Scintillation Counter (Hidex Oy, Finland). The recovery of  $^{33}\text{P}$  (%) in a specific P pool was calculated as:

$$^{33}\text{P recovery (\%)} = (r/R) \times 100, \quad [2]$$

where  $r$  and  $R$  is the radioactivity ( $\text{Bq g}^{-1}$  soil) in the extracted pool and the total amount of added  $^{33}\text{P}$  activity, respectively (Bünemann et al. 2004). The relative specific activity (SA) was calculated as:

$$\text{rel. specific activity (\%)} = (r/R) / Q_p, \quad [3]$$

where  $r$  and  $R$  is the radioactivity ( $\text{Bq g}^{-1}$  soil) in the extracted pool and the total amount of added  $^{33}\text{P}$  activity, respectively.  $Q_p$  is the amount of P ( $\text{mg P kg}^{-1}$  soil) in a given pool.

The recovery of  $^{33}\text{P}$  in  $P_{\text{mic}}$  was corrected for the effect of sorption, isotopic exchange and extraction efficiency.  $^{33}\text{P}$  recovery in  $P_{\text{mic}}$  was calculated as:

$$^{33}\text{P}_{\text{mic}} = (^{33}\text{P}_{\text{fumigated}} - ^{33}\text{P}_{\text{unfumigated}}) / K_p \quad [4]$$

where  $K_p = 0.69$  is the correction factor to account for the effect of sorption, isotopic exchange and extraction efficiency as mentioned above.

### 2.1.3.6 Data analysis

The results given are arithmetic means of four replicates in each treatment and expressed on an oven-dry weight basis. Normality and homogeneity of variance were checked using Shapiro-Wilk's  $W$  test and Levene tests,



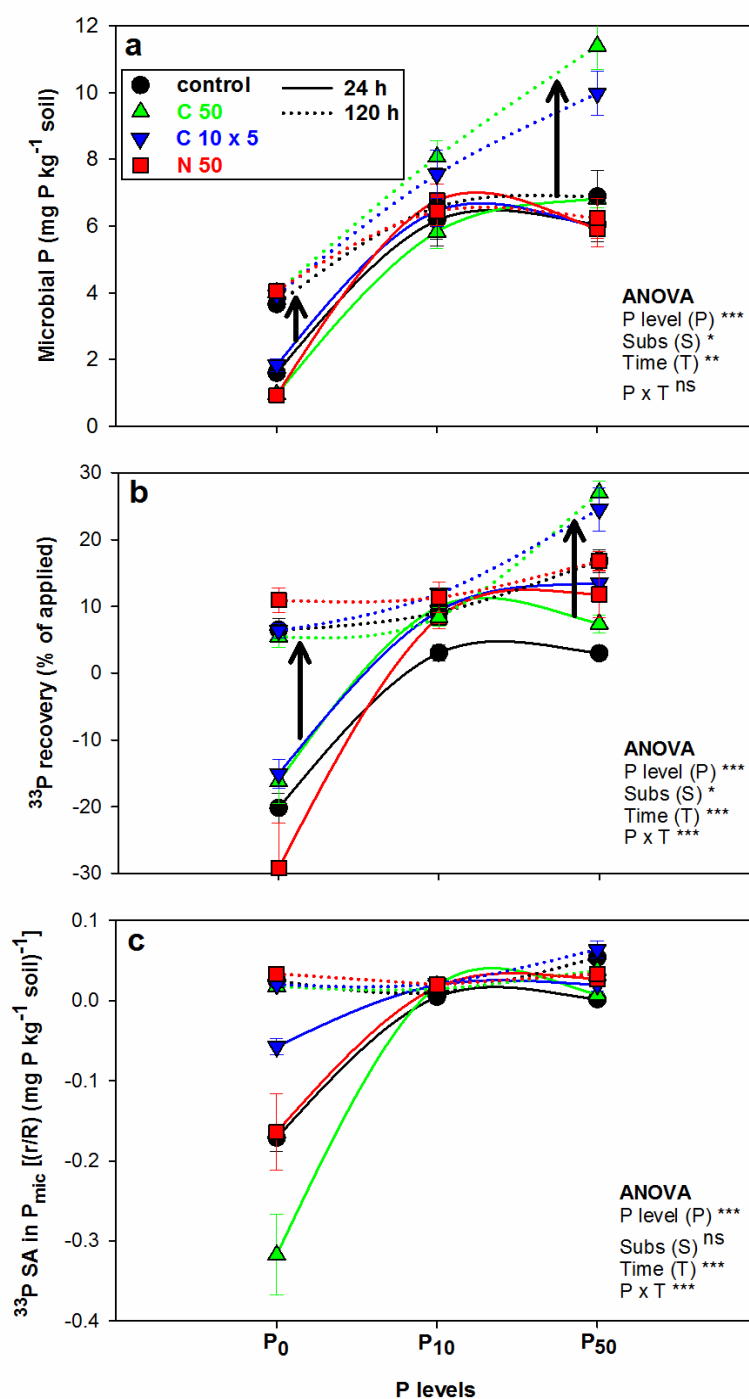
respectively. For each sampling time (24 h and 120 h) of the incubation experiment, data were tested by two-way analysis of variance (ANOVA) with (3) P levels and (4) substrates as the factors. Interaction between P levels x substrates was tested. Data were also subjected to three-way (ANOVA) with (3) P levels, (4) substrates and (2) sampling times as a factors. All possible interactions were also tested. Multiple comparisons (all-pairwise comparisons) using Tukey's test were performed whenever the ANOVA indicated significant differences at  $P \leq 0.05$ . Relationships between P pools were evaluated using Multiple Linear Regression. All statistical analyses were carried out using STATISTICA 12 (StatSoft Inc., USA).

### 2.1.4 Results

#### 2.1.4.1 Microbial P pool

Microbial biomass P ( $P_{mic}$ ) was higher ( $P < 0.05$ ) after adding labile P as  $KH_2PO_4$ , regardless of the level (either low ( $P_{10}$ ) or high ( $P_{50}$ )) compared to no P (Fig. 1a; Table 2).  $P_{mic}$  increases ( $P < 0.05$ ) exponentially after P addition by about 3-fold compared to soil with no P after 24 h. The average difference recorded between  $P_0$  and  $P_{10}$  was  $5.1 \text{ mg P kg}^{-1}$  soil and remained constant for  $P_0$  and  $P_{50}$ . After 120 h, however,  $P_{mic}$  in  $P_0$  slightly increased ( $P < 0.05$ ), which resulted in a smaller difference ( $3.9 \text{ mg P kg}^{-1}$  soil) compared to  $P_{10}$  and  $P_{50}$ . Nonetheless, the increase in  $P_0$  across all soils was not identical to those recorded in  $P_{10}$  and  $P_{50}$  and did not even reach the 24 h  $P_{mic}$  content of soils with P addition. In contrast,  $P_{10}$  and  $P_{50}$  had more or less the same  $P_{mic}$  content across all soils after 24 h, with an average of  $6.0 \text{ mg P kg}^{-1}$  soil (Fig. 1a). This reveals that microorganisms were more rapidly saturated with P even at low P addition ( $P_{10}$ ); therefore, a constant  $P_{mic}$  content was recorded. The soil amended with C and N behaved similarly to control soil after 24 h at all P levels, showing that substrate addition did not influence microbial uptake of P during the early incubation period. However, after 120 h, C addition affected microbial P uptake in the soil regardless of the method of glucose application, i.e. either one-time addition ( $C_{50}$ ) or staggered ( $C_{10 \times 5}$ ) (Fig. 1a). The effects were greater ( $P < 0.05$ ) in soil with the highest P level ( $P_{50}$ ), where  $P_{mic}$  was about  $9.5 \text{ mg P kg}^{-1}$  soil and almost twice the content at 24 h. This

reveals that soil microorganisms were limited by C, which affects microbial growth and activities such as P uptake. Indeed, microbial biomass C increased after C addition (Fig. S1 Supplementary Material).



**Figure 1** (a) Phosphorus content, (b) <sup>33</sup>P recovery and (c) relative specific activity (SA) in microbial phosphorus (P<sub>mic</sub>) after the addition of <sup>33</sup>P tracer alone (P<sub>0</sub>), <sup>33</sup>P-labeled fertilizer as KH<sub>2</sub>PO<sub>4</sub> applied to soil as 10% (P<sub>10</sub>) and 50% (P<sub>50</sub>) of the initial P content and combined with substrates: i.e. distilled water as control, glucose and ammonium nitrate as carbon and nitrogen source, respectively. Bars indicate standard error of four replicates. Arrows indicate significant increase or decrease between 24 and 120 h

$^{33}\text{P}$  recovery in  $P_{\text{mic}}$  was greater ( $P < 0.05$ ) after addition of labile P (either  $P_{10}$  or  $P_{50}$ ) than without P addition ( $P_0$ ), in which the  $^{33}\text{P}$  recovered was negative after 24 h (Fig. 1b). An average of 9.2 and 11.0% of applied  $^{33}\text{P}$  were recovered in soils amended with C and N in  $P_{10}$  and  $P_{50}$ , respectively. These values were 3 to 4 times higher than in soils which received distilled water only. Therefore, C and N addition affect microbial P uptake, as noted above.

**Table 2** Main treatment effects on  $^{33}\text{P}$  recovery and phosphorus content in the P pools after sequential fractionation

Factor	Recovery of $^{33}\text{P}$ (% of applied)			Phosphorus content (mg P kg <sup>-1</sup> soil)		
	$P_{\text{mic}}$	$P_{\text{AEM}}$	$P_{\text{NaOH}}$	$P_{\text{mic}}$	$P_{\text{AEM}}$	$P_{\text{NaOH}}$
<b>P level (P)</b>						
0	6.43c <sup>§</sup>	15.0b	34.7b	2.56b	6.08c	27.8a
10%	8.96b	33.4a	44.1a	6.71a	13.9b	27.5a
50%	15.1a	33.5a	42.3a	6.93a	28.1a	20.0b
<b>Substrate (S)</b>						
H <sub>2</sub> O	3.05c	29.1a	40.1ns <sup>‡</sup>	4.63b	16.3ns	24.4ns
C50	7.01a	26.4ab	4.34ns	6.84a	15.8ns	25.1ns
C10 x 5	8.45a	26.2b	39.9ns	5.41ab	15.5ns	25.4ns
N50	5.03b	27.7ab	40.9ns	4.72b	16.6ns	25.4ns
<b>Time (T)</b>						
24 h	1.18b	35.4a	38.0b	4.50b	20.4a	23.1b
120 h	13.0a	19.2b	42.7a	6.30a	11.7b	27.0a
<b>Significance of Interactions</b>						
P x S	ns	ns	ns	ns	ns	ns
P x T	***	***	ns	ns	***	*
S x T	ns	ns	ns	ns	ns	ns
P x S x T	ns	ns	ns	ns	ns	ns

\* $P < 0.05$ ; \*\*\*  $P < 0.001$

§ Means within columns and factors followed by the same letter are not significantly different ( $P = 0.05$ ) by Tukey's multiple range test.

‡ ns = not significant

After 120 h, the  $^{33}\text{P}$  recovered in soils with no P ( $P_0$ ) greatly increased ( $P < 0.05$ ), averaging 7.3% of applied  $^{33}\text{P}$  across all soils. The increase ( $P < 0.05$ ) in  $P_0$  across all soils after 120 h more or less reached the mean level of  $^{33}\text{P}$  recovery in  $P_{10}$  and  $P_{50}$  for 24 h and 120 h (Fig. 1b). In contrast,  $P_{10}$  and  $P_{50}$  had a constant level of recovered  $^{33}\text{P}$ , except for soils with C addition in  $P_{50}$ , which increased the content recovered after 24 h by almost twice (Fig. 1b). Furthermore, an average of 10.0% of the applied  $^{33}\text{P}$  recovered across all

soils went to the microbial biomass pool after 120 h (Fig. 1b); this value increased to as much as 26% if C was present ( $P_{50}$ ; Fig. 1b). This reveals that microorganisms are an important factor affecting the P dynamics in soils.

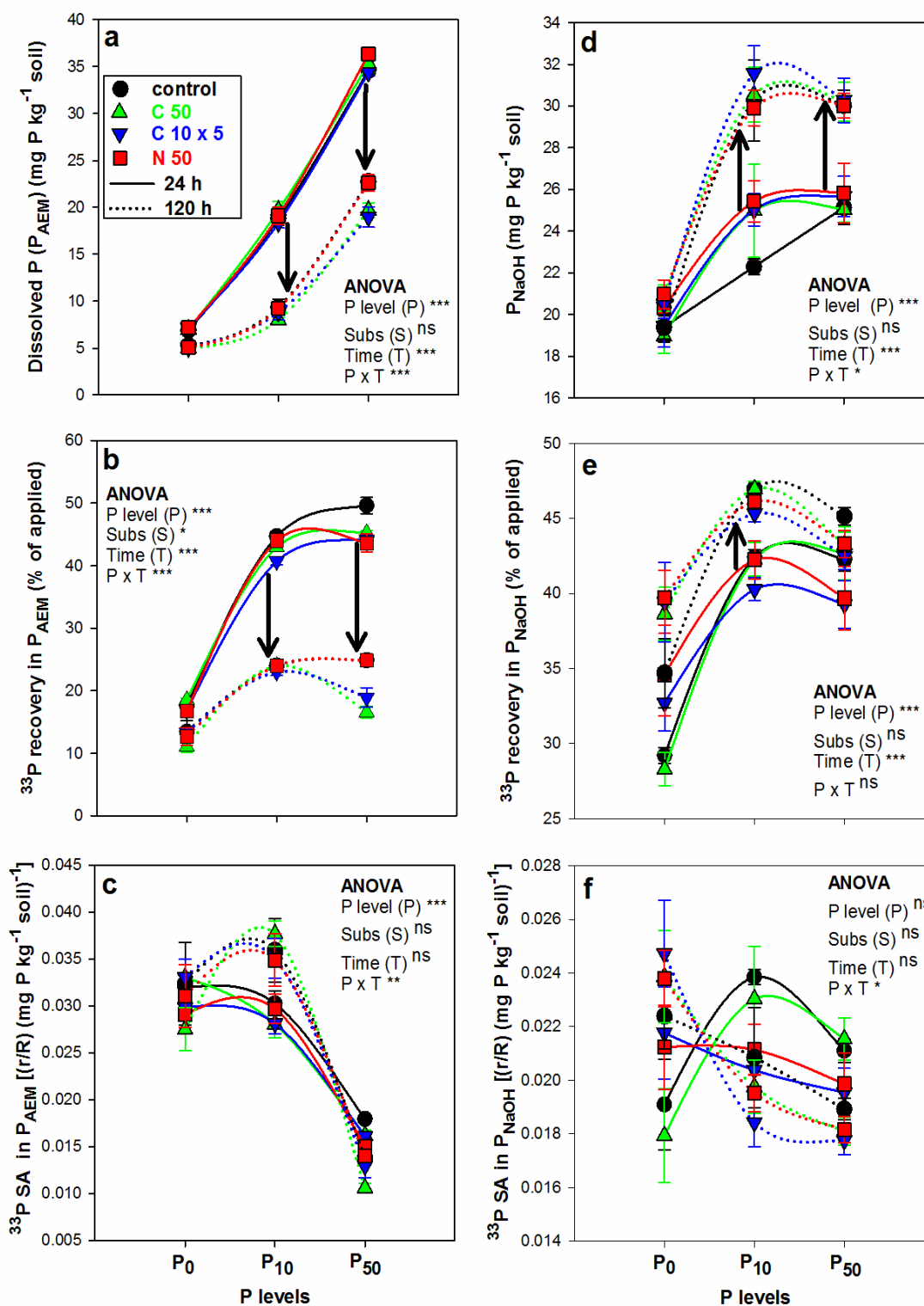
### 2.1.4.2 Available P pool ( $P_{AEM}$ )

We recorded a clear trend in available P content ( $P_{AEM}$ ) as related to ( $P < 0.05$ ) the amount of P and to incubation time (Fig. 2a; Table 2).  $P_{AEM}$  was always greater ( $P < 0.05$ ) in soils with a higher amount of labile P addition ( $P_0 < P_{10} < P_{50}$ ) throughout the incubation. In 24 h, the average difference between  $P_0$  and  $P_{10}$  was 11.9 mg P kg<sup>-1</sup>, whereas the difference was 28.1 mg P kg<sup>-1</sup> between  $P_0$  and  $P_{50}$  across all soils (Fig. 2a). Nonetheless, the  $P_{AEM}$  content decreased ( $P < 0.05$ ) by about 27%, 52% and 40% in  $P_0$ ,  $P_{10}$  and  $P_{50}$ , respectively, after 120 h. We hypothesized that a decreased  $P_{AEM}$  content after 120 h was primarily due to the distribution of labile P to other pool(s) as a consequence of microbial P uptake and P sorption by Fe and/or Al oxides present in the soil.

The percentage recovery of applied <sup>33</sup>P in the  $P_{AEM}$  pool was almost the same as in the  $P_{NaOH}$  pool for  $P_{10}$  and  $P_{50}$  in the first 24 h (Fig. 3). Recovery was always higher ( $P < 0.05$ ) at 24 h – by as much as 45% of applied <sup>33</sup>P – compared to 120 h for all P levels. After 120 h, <sup>33</sup>P recovery decreased ( $P < 0.05$ ), and this drop was very pronounced in  $P_{10}$  and  $P_{50}$  compared to  $P_0$  (Fig. 2b). The decrease was about half of the <sup>33</sup>P recovered at 24 h. This indicates that available P was redistributed faster to another P pool(s) within 120 h. The relative SAs in  $P_{AEM}$  decreased ( $P < 0.05$ ) because the rate of P addition increased during the incubation period (Fig. 2c).

### 2.1.4.3 Poorly available P pool ( $P_{NaOH}$ )

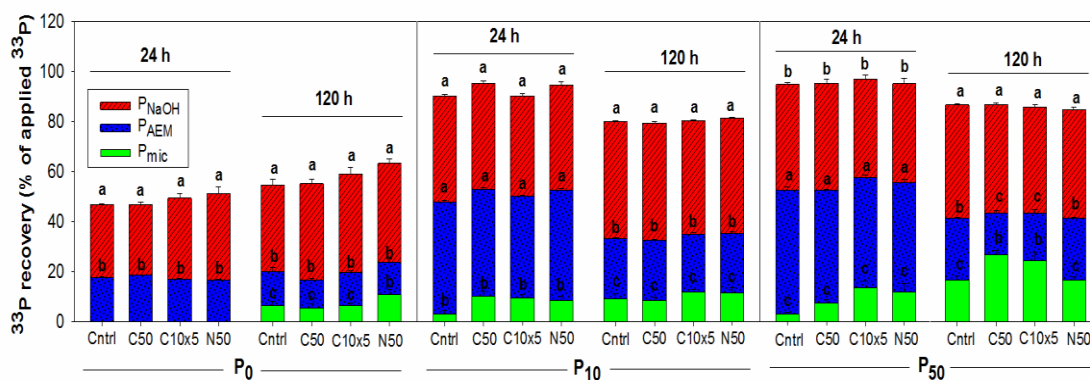
The P pool extracted with 0.1 M NaOH corresponds to the P associated with Al and Fe oxides in the acidic soil (Hedley et al. 1982; Maranguit et al. 2017). This P pool involves long-term release and acts as a very slow buffer for labile P in acidic P-limited soils. Like  $P_{mic}$  and  $P_{AEM}$ , the P content in  $P_{NaOH}$  increased ( $P < 0.05$ ) after  $P_{10}$  and  $P_{50}$  addition compared to  $P_0$ , with an average increase across all soils of 4.9 and 5.9 mg P kg<sup>-1</sup> soil, respectively



**Figure 2** Phosphorus content,  $^{33}\text{P}$  recovery and relative specific activity (SA) in available P ( $P_{AEM}$ ) (a-c, respectively) and Fe/Al-bound P ( $P_{NaOH}$ ) (d-f, respectively) after the addition of  $^{33}\text{P}$  tracer alone ( $P_0$ ),  $^{33}\text{P}$ -labeled fertilizer as  $\text{KH}_2\text{PO}_4$  applied to soil as 10% ( $P_{10}$ ) and 50% ( $P_{50}$ ) of the initial P content and combined with substrates: i.e. distilled water as control, glucose and ammonium nitrate as carbon and nitrogen source, respectively. Bars indicate standard error of four replicates. Arrows indicate significant increase or decrease between 24 and 120 h. Note the different scale of the y-axis (a & d; b & e; c & f)

(Fig. 2d). Nonetheless, during the first 24 h, less added P was incorporated into the  $P_{\text{NaOH}}$  pool, showing less difference of  $P_{10}$  and  $P_{50}$  to  $P_0$ . After 120 h, however, a large increment ( $P < 0.05$ ) – twice the increase as at 24 h – was recorded in soils with both low and high P addition. Nonetheless, the increase in  $P_{10}$  and  $P_{50}$  across all soils was almost the same, ranging from 9 to 11 mg P  $\text{kg}^{-1}$  soil (Fig. 2d). The increasing  $P_{\text{NaOH}}$  content after 120 h supports our hypothesis on available P ( $P_{\text{AEM}}$ ), i.e. that part of it was distributed to the less available pool such as  $P_{\text{NaOH}}$  after 120 h.

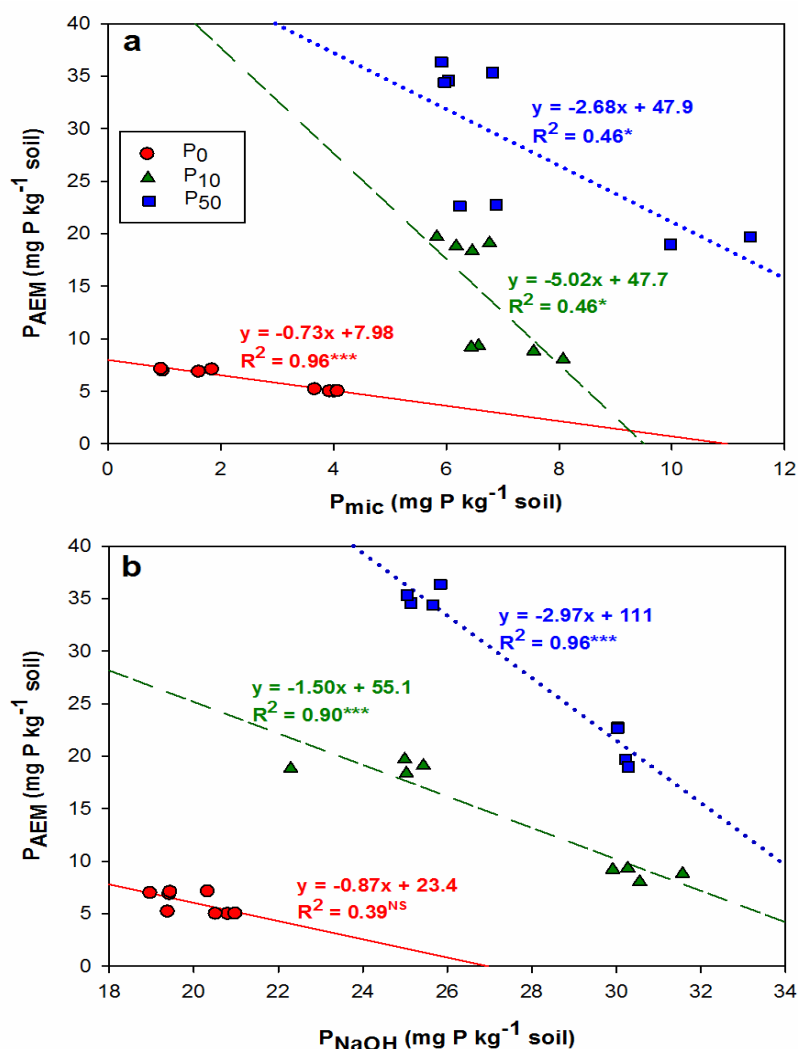
Finally, the proportion of  $^{33}\text{P}$  recovered in  $P_{\text{NaOH}}$ , the average ranging from 28 to 42% of the applied  $^{33}\text{P}$ , was almost the same as that in  $P_{\text{AEM}}$  in the first 24 h (Fig. 3). However, the proportion changed after 120 h due to the re-distribution of  $^{33}\text{P}$ -labeled fertilizer; this re-distribution caused a decline of  $P_{\text{AEM}}$  and an increase of  $P_{\text{NaOH}}$  (Fig. 2b, 2e & 3). Indeed, a slight increase of  $^{33}\text{P}$  recovered in  $P_{\text{NaOH}}$  ranging from 1-10% of the applied  $^{33}\text{P}$  after longer incubation time was recorded across all soils (Fig. 2e).  $^{33}\text{P}$  recovery in  $P_{10}$  and  $P_{50}$  had almost the same percentage in 24 h and in 120 h. The relative SA generally dropped after the higher P rate application and decreased throughout incubation period (Fig. 2f).



**Figure 3** Total  $^{33}\text{P}$  recovery by sequential extraction of soil after the addition of  $^{33}\text{P}$  tracer alone ( $P_0$ ), 10% ( $P_{10}$ ) and 50% ( $P_{50}$ ) of initial P content using  $\text{KH}_2\text{PO}_4$  labeled with  $^{33}\text{P}$  to soils with combined application of glucose and ammonium nitrate as carbon and nitrogen source, respectively. Letters in each column indicate differences between P pools ( $P < 0.05$ ) based on Tukey's multiple range tests.

#### 2.1.4.4 Relationship between P pools

The P pools were interrelated with each other. A strong negative relationship ( $P < 0.05$ ) between  $P_{mic}$  and  $P_{AEM}$  – with  $R^2$  ranging from 0.46-0.96 across all P levels – was recorded (Fig. 4a). Likewise, a strengthened negative relationship was found between  $P_{NaOH}$  and  $P_{AEM}$  with increasing P amendment ( $P_0 < P_{10} < P_{50}$ ;  $R^2 = 0.39^{ns} < 0.90^{***} < 0.96^{***}$ ; Fig. 4b). The correlation between  $P_{NaOH}$  and  $P_{AEM}$  was much more pronounced than that between  $P_{mic}$  and  $P_{AEM}$  in the P-amended soils. Therefore, the fast decline of  $P_{AEM}$  is strongly associated with P adsorption by Fe and Al oxides and to a lesser extent with microbial P uptake.

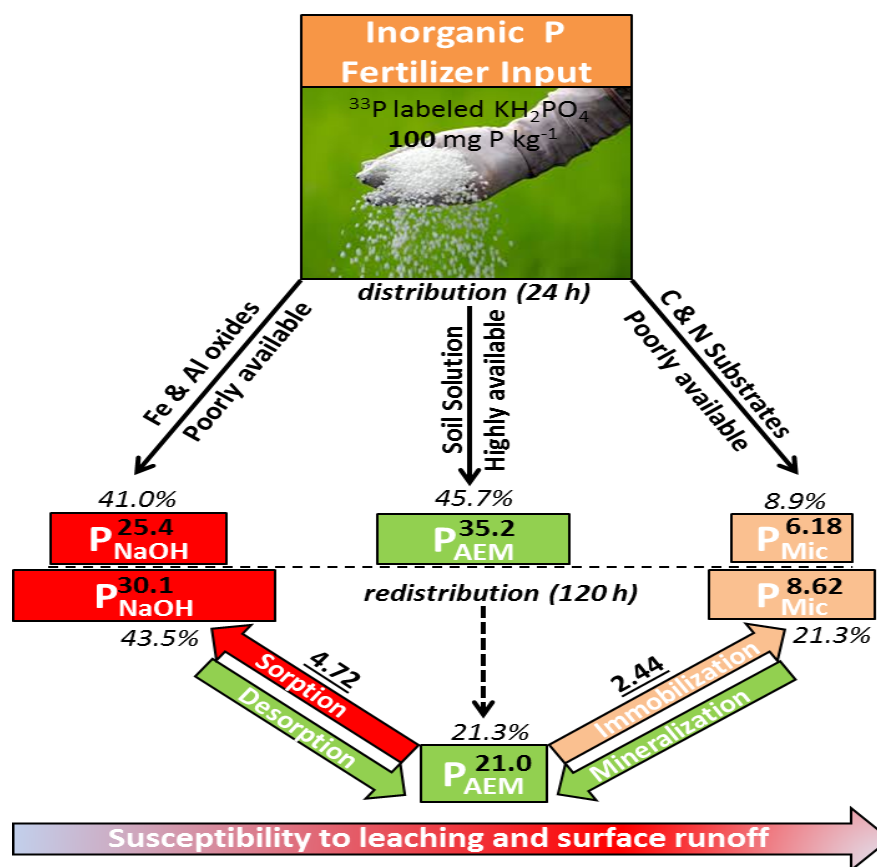


**Figure 4** Linear relationship between phosphorus pools: (a)  $P_{mic}$  and  $P_{AEM}$ ; (b)  $P_{NaOH}$  and  $P_{AEM}$ . Each point corresponds to the mean of four replicates

## 2.1.5 Discussion

### 2.1.5.1 Distribution of P among fractions and dynamics over time

Tracing the fate and distribution of added P to various pools was simple and fast using  $^{33}\text{P}$ -labeled phosphate. Inorganic P fertilization changes the size of soil P pools (Fig. 5) and also reveals a distinct temporal pattern in the P pool content over the 120 h incubation period (Table 2). Basically, the high rate of P application yielded the greatest available P content ( $\text{P}_{\text{AEM}}$ ) in the soil compared to low P ( $\text{P}_{10}$ ) and no P addition ( $\text{P}_0$ ) (Fig. 2a). Importantly, not all the applied P remains in the available pool for plant uptake.



**Figure 5** The fate of inorganic phosphorus applied to acidic P-limited soil. The distribution of P fertilizer in P pools is affected by biological and physicochemical reactions. Box size indicates the proportion of P fertilizer recovered in each pool throughout the incubation period. The susceptibility of each pool to leaching and surface run-off is determined by the strength of binding in the soil. The rate of susceptibility is indicated by the intensity of the red color in the arrow (e.g., intense red = highly susceptible). Values in bold inside the box: P content in respective pool (mg P kg<sup>-1</sup>); values in italics:  $^{33}\text{P}$  recovery in respective pool (% of applied  $^{33}\text{P}$ ); underlined values: the total increase of P content (mg P kg<sup>-1</sup>) immobilized and adsorbed at 120 h. Values above and below horizontal dashed line represent contents at 24 h and 120 h, respectively.



Instead, it is rapidly distributed to less available pools: both high and low P application resulted in strong P immobilization by microorganisms and fixation by Fe and Al oxides, as is evident in the elevated  $P_{mic}$  (Fig. 1a) and  $P_{NaOH}$  (Fig. 2d) content compared to no P addition. Nonetheless, these pools are very important P reserves; buffering available P.

$^{33}P$  recovery in  $P_{AEM}$  (Fig. 2b) was in accordance with the principles of isotopic exchange (Fardeau 1996; Bünemann et al. 2004): it diminished steadily, with the greatest decrease at 120 h. Our findings agree with the trends observed during the incubation of temperate soils amended with  $^{33}P$ -labeled soybean residues (Daroub et al. 2000), in highly weathered Oxisols from Colombia (Bühler et al. 2002) and in kaolinitic Oxisols from Kenya (Bünemann et al. 2004). Furthermore, a significant fraction of the added labeled P was irreversibly fixed in soils that sorb very high P amounts, thereby reducing the  $^{33}P$  fraction actually participating in the isotopic exchange (Wolf et al. 1986). In our study, the lower  $^{33}P$  recovery in  $P_{AEM}$  (Fig. 2b) was accompanied by a simultaneous increase in the  $P_{mic}$  (Fig. 1b) and  $P_{NaOH}$  (Fig. 2e) after 120 h of incubation. This reverse trend of  $^{33}P$  recovered in different P pools after 120 h suggests that  $^{33}P$ -labeled phosphate was distributed from the labile pool ( $P_{AEM}$ ) to the immobilized pool by microorganisms ( $P_{mic}$ ) and by mineral sorption ( $P_{NaOH}$ ).

The  $^{33}P$  recovered in  $P_{mic}$  after 120 h – with a strong increase at  $P_0$  compared to no increase at  $P_{10}$  and less increase in  $P_{50}$  (Fig. 1b) – can be explained by the microorganism response to labile P. We hypothesized that microorganisms fully trap the limiting resource (P) until P becomes totally restricted. During the first 24 h at  $P_0$ , microorganisms were actually P-limited and not fully activated even though the addition of C and N led to less consumption and saturation of  $^{33}P$  in the microbial biomass. After the longer incubation period (120 h), the increasing microbial activity and growth resulted in greater ( $P < 0.05$ )  $^{33}P$  recovery. In  $P_{10}$ , although labile P was increased only minimally (e.g. 10% of initial P content ( $0.2 \text{ mg g}^{-1}$ )), it was apparently enough for microorganisms to become activated and saturated with P during the first 24 h. These results suggest that, when new labile P is applied in the system,

P can be rapidly immobilized by soil microorganisms under limiting P conditions (Bünemann et al. 2012). Accordingly, the P concentration in the soil solution is strongly influenced by microbial P immobilization (Frossard et al. 2000). We hypothesized that, after being saturated with P and initial stimulation, microorganisms return to dormancy. Our results support this hypothesis, explaining why the  $^{33}\text{P}$  recovered in the microbial biomass has been found to be rather constant over time (Bünemann et al. 2004, 2012; Oberson et al. 2001). This increases the chance for the remaining label fertilizer to be adsorbed/fixed by Fe/Al oxides, which is reflected in the slight increase of  $^{33}\text{P}$  recovered (Fig. 2e) and of the P content (Fig. 2d) in the  $\text{P}_{\text{NaOH}}$  pool after 120 h. A longer incubation study (34 days) showed a subsequent movement of the label from the labile pool to the  $\text{P}_{\text{NaOH}}$  pool (Daroub et al. 2000).

In contrast to  $\text{P}_0$  and  $\text{P}_{10}$ ,  $\text{P}_{50}$  almost tripled the labile P content of the soil available for microorganisms and for plant-uptake. This explains the higher microbial activity even during the first 24 h, which led to the greatest  $^{33}\text{P}$  recovery in microbial biomass (Fig. 1b). Nonetheless, the  $^{33}\text{P}$  recovered in  $\text{P}_{10}$  and  $\text{P}_{50}$  was almost the same in all soils throughout the incubation time, except for soils amended with glucose as a source of C. Therefore, microorganisms will be easily saturated at a certain P level regardless of how high the available P content is in the soil. Consequently, in  $\text{P}_{50}$ , part of the  $^{33}\text{P}$ -labeled fertilizer not taken-up by microorganisms went to the  $\text{P}_{\text{NaOH}}$  pool. Likewise, free Fe and Al for P binding also had a saturation point. This explains the more or less identical P content and  $^{33}\text{P}$  recovery in the  $\text{P}_{\text{NaOH}}$  pool in soil with  $\text{P}_{10}$  and  $\text{P}_{50}$  (Fig. 2e). In the experiment without plants, the excess of label fertilizer in soils with  $\text{P}_{50}$  remains in the labile pool, free to be accessed again by microorganisms. Under natural conditions, however, competition between plant and microorganisms will influence the availability and depletion of available P. At any rate, the synthesis of microbial biomass is stimulated by adding any substrate as a C source (Ayaga et al. 2006; Spohn and Kuzyakov 2013). In fact, very small amounts of labile C substrate ( $5\text{-}15 \mu\text{g g}^{-1}$ ) can activate soil microorganisms (De Nobili et al. 2001; Mason-Jones

and Kuzyakov 2017). This increases the demand for P, boosting the recovery of label in the  $^{33}\text{P}$  microbial pool (Bünemann et al. 2004). Therefore, microorganisms took up the remaining labile P from solution, and  $^{33}\text{P}$  recovery increased in the  $P_{\text{mic}}$  pool in soils amended with C in  $P_{50}$  after 120 h (Fig. 1b; Fig. S2 Supplementary Material). A high  $^{33}\text{P}$  recovery of 66% was recorded in chloroform-labile P after 2 d when soils were amended with glucose and ammonium nitrate, compared with 8% in the absence of easily available sources of C and N (Oehl et al. 2001).

In our study, 86% of the total applied  $^{33}\text{P}$  was recovered by the sequential fractionation after 120 h (Fig. 3). Consequently, the unrecovered label is incorporated into non-extractable pools.

### *2.1.5.2 P availability and ecological relevance of microbial P and the $P_{\text{NaOH}}$ pool*

The negative relationships between  $P_{\text{mic}}$  and  $P_{\text{AEM}}$  ( $P < 0.05$ ;  $R^2 = 0.46-0.53$ ; Fig. 4a) after labile P addition and the significant recovery of  $^{33}\text{P}$  in microbial biomass (Fig. 1b) indicates that P availability in the soil solution is influenced by microorganisms. Our findings support the hypotheses that microbial P utilization and mobilization are important for improving the synchrony between plant nutrient demands and the P-supplying capacity of high-P-fixing soils (Picone et al. 2003; Richardson and Simpson 2011; Koutika et al. 2013). The addition of P to P-limited soils, coupled with adding organic matter as a substrate for microorganisms, will boost microbial growth and activity (De Nobili et al. 2001; Ayaga et al. 2006; Spohn and Kuzyakov 2013). The result is that significant amounts of P are incorporated in microbial cells. The significant microbial P pool in our study (Fig. 1a), coupled with the rapid turnover time of soil microbial P, suggest that it is an increasingly important source of plant-available phosphorus as soils age and become P-limited (Brookes et al. 1984; Kouno et al. 2002). Likewise, the significant amount of  $^{33}\text{P}$  recovered in the  $P_{\text{NaOH}}$  pool (Fig. 2e & 3) and the strong negative relationship between  $P_{\text{NaOH}}$  and  $P_{\text{AEM}}$  ( $P < 0.001$ ;  $R^2 = 0.90-0.96$ ; Fig. 4b) reflect the importance of P sorption on sesquioxides (Bünemann et al. 2004). This buffers the available P supply (Hedley et al. 1982; Maranguit et al. 2017).

At the same time, we observed significant depletion of the  $P_{AEM}$  fraction. Although the transformation of this pool to the labile pool requires a long time, the long-term release of the  $P_{NaOH}$  reserve is very important. It protects P from leaching and surface run-off and potentially buffers available P for plant-uptake. According to our results (Fig. 5), the amounts of P in various pools measured by sequential P extraction and the fluxes of P between pools are controlled by several mechanisms: (1) physicochemical, i.e. sorption/desorption (Turner et al. 2013), which is more pronounced in highly acidic soil, which has a high P fixing ability, and (2) biological/biochemical reactions, i.e. immobilization/mineralization (Turner et al. 2013; Bünemann et al. 2016), which are very important in retaining P and returning it back to the soil after a fast turnover. These results have important implications for the development of sustainable management systems, especially in light of the impending depletion of rock phosphate reserves for fertilizer production worldwide, and for fertilizer-use efficiency (Cordell et al. 2009).

### 2.1.6 Conclusions

We traced the fate and the rate of distribution of labile P to various pools using the  $^{33}P$  isotope labeling technique. Our experimental results confirmed our first hypothesis that the amounts of P in various pools (measured by sequential P extraction procedures) and the fluxes of P between pools are controlled both by physicochemical factors, i.e. sorption/desorption, and by biological reactions, i.e. immobilization/mineralization processes. When P fertilizers are applied to soil, phosphate ions are released into the solution, followed by a fast and almost instantaneous fixation by the Fe and Al oxides and immobilization by microbial uptake. The extent to which P remains in the soil solution depends on the degree to which it is adsorbed, desorbed and mineralized. High P application (50% of the initial total P) enhanced the available content ( $\approx 35$  mg P  $kg^{-1}$  soil) for plant-uptake, but only for a short time. Applied P, if not directly taken-up by plants, will be distributed quickly to less available pools. This proves our second hypothesis. Indeed, both high and low P application led to strong P immobilization by microorganisms ( $\approx 10$ -20% of applied  $^{33}P$ ), and fixation by Fe and Al oxides ( $\approx 45\%$  of applied  $^{33}P$ )

caused the amount of available P to drop. Applying glucose as a C source boosts the microbial activity, growth and demand for P, which increases the microbial biomass P pool ( $\approx 10 \text{ mg P kg}^{-1} \text{ soil}$ ). This confirms our third hypothesis. Nonetheless, the turnover of microbial biomass P and desorption of fixed P sustains long-term P fertility. Furthermore, P fixation by Fe and Al oxides also important in keeping the P in the soil system and protects it from leaching and from surface run-off.

### 2.1.7 Acknowledgement

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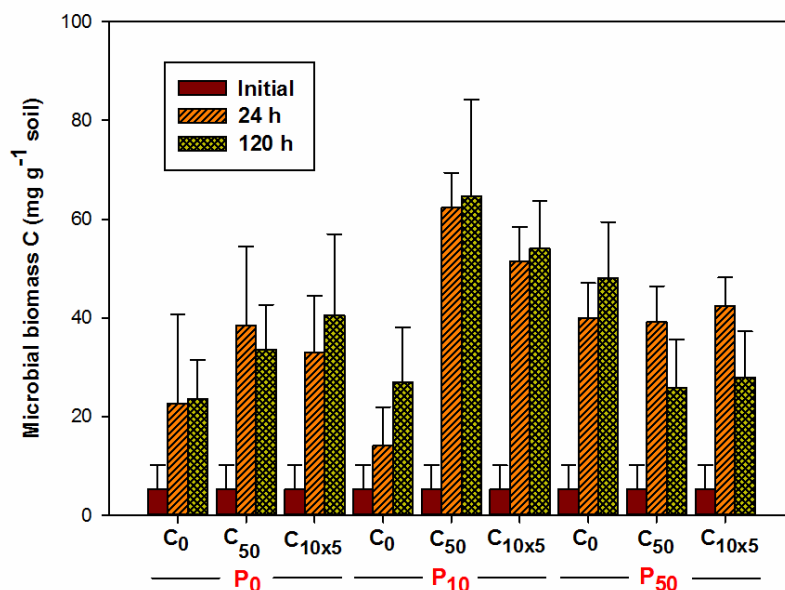
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## 2 Publications and Manuscripts: Study 1

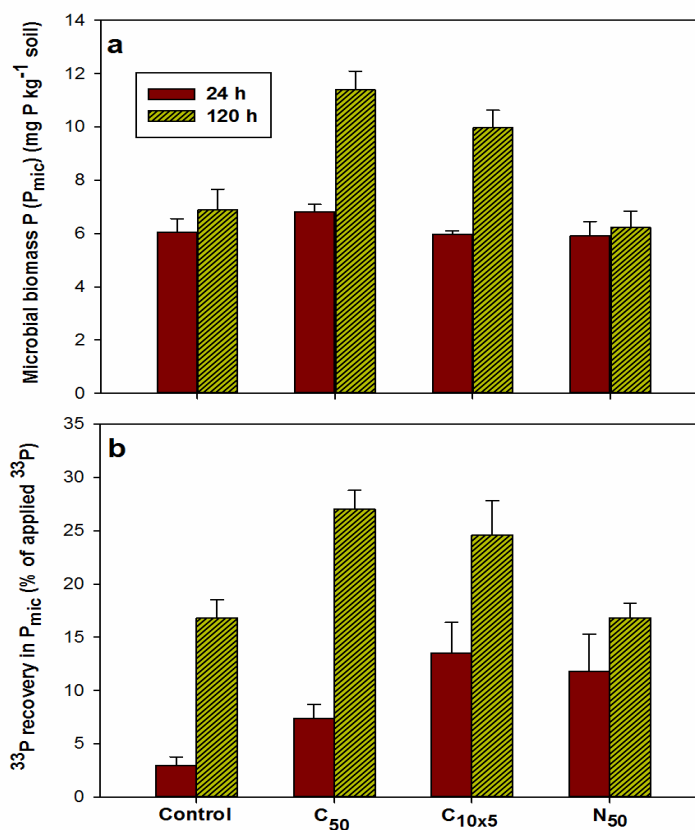
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## 2.1.9 Supporting Information



**Fig. S1** Microbial biomass carbon (MBC) after addition of <sup>33</sup>P tracer alone (P<sub>0</sub>), <sup>33</sup>P-labeled fertilizer as KH<sub>2</sub>PO<sub>4</sub> applied to soil as 10% (P<sub>10</sub>) and 50% (P<sub>50</sub>) of the initial P content and combined with substrates: i.e. distilled water as control and glucose as carbon source. Bars indicate standard error of four replicates



**Fig. S2** (a) Microbial biomass phosphorus (P<sub>mic</sub>) content and (b) <sup>33</sup>P recovered in P<sub>mic</sub> pool in soils with <sup>33</sup>P-labeled fertilizer as KH<sub>2</sub>PO<sub>4</sub> applied as 50% of the initial P content - depending on the presence of carbon and nitrogen sources applied as glucose and ammonium sulfate, respectively. Bars indicate standard error of four replicates



### 2.2 Study 2: Land-use change affects phosphorus fractions in highly weathered tropical soils

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#### 2.2.1 Abstract

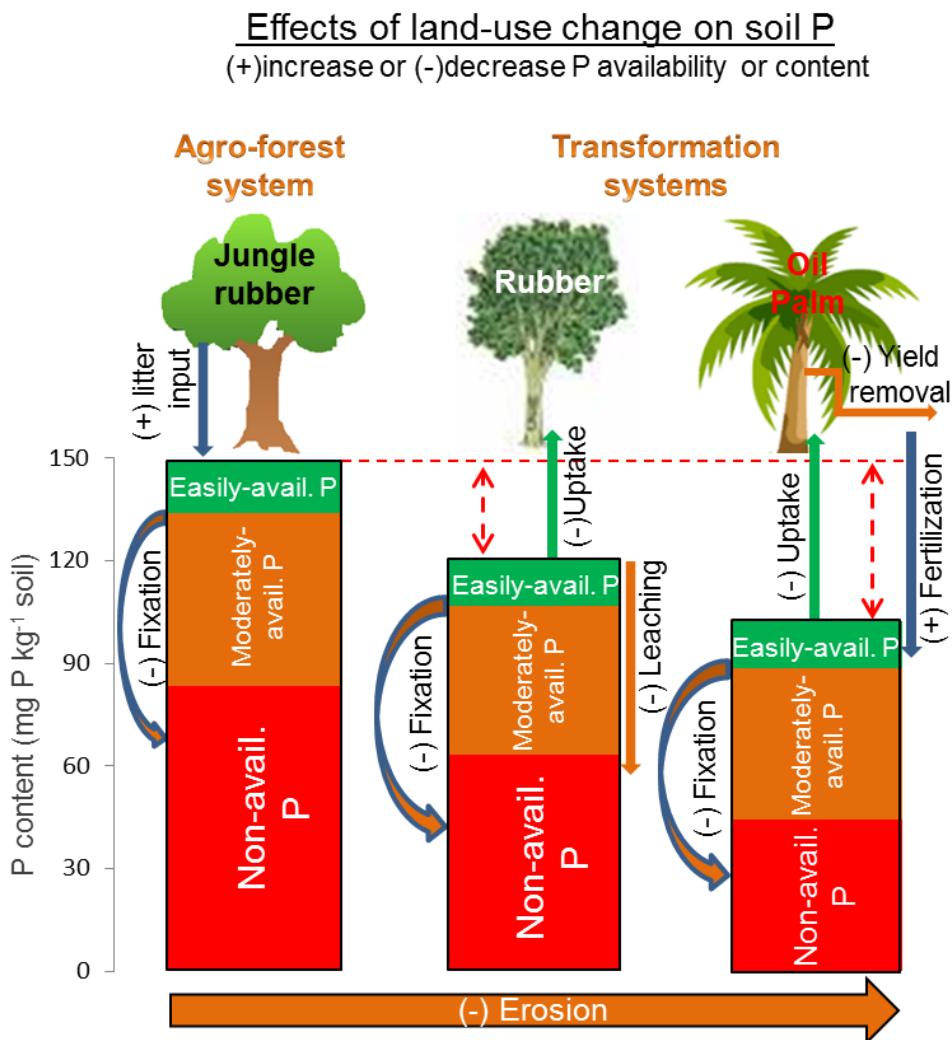
Deforestation and land-use change in tropics have increased over the past decades, driven by the demand for agricultural products. Although phosphorus (P) is one of the main limiting nutrients for agricultural productivity in the tropics, the effect of land-use change on P availability remains unclear. The objective was to assess the impacts of land-use change on soil inorganic and organic P fractions of different availability (Hedley sequential fractionation) and on P stocks in highly weathered tropical soils. We compared the P availability under extensive land-use (rubber agroforest) and intensive land-use with moderate fertilization (rubber monoculture plantations) or high fertilization (oil palm monoculture plantations) in Indonesia. The P stock was dominated by inorganic forms (60 to 85%) in all land-use types. Fertilizer application increased easily-available inorganic P (i.e.,  $H_2O\text{-Pi}$ ,  $NaHCO_3\text{-Pi}$ ) in intensive rubber and oil palm plantations compared to rubber agroforest. However, the easily-available organic P ( $NaHCO_3\text{-extractable Po}$ ) was reduced by half under oil palm and rubber. The decrease of moderately available and non-available P in monoculture plantation means that fertilization maintains only the short-term soil fertility that is not sustainable in the long run due to the depletion of P reserves. The mechanisms of this P reserve depletion are: 1) soil erosion (here assessed by C/P ratio), 2) mineralization of soil organic matter (SOM) and 3) P export with yield products. Easily-available P fractions (i.e.,  $H_2O\text{-Pi}$ ,  $NaHCO_3\text{-Pi}$  and  $Po$ ) and total organic P were strongly positively correlated with carbon content, suggesting that SOM plays a

key role in maintaining P availability. Ecologically based management is therefore necessary to mitigate SOM losses and thus increase the sustainability of agricultural production in P-limited, highly weathered tropical soils.

**Key words:** Land-use change, Rainforest deforestation, Phosphorus fractions and stocks, Carbon-to-Phosphorus ratio, Hedley fractionation, Erosion consequences, Agroforestry

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**Graphical Abstract**



### 2.2.2 Introduction

Land-use change and intensification of cultivation are the predominant global changes of this century. This is mainly because of the global socio-economic demand for food, feed, fiber and biofuel driven by population growth (Geissen et al., 2009; Guillaume et al., 2015). Intensification of agriculture involving high-yielding crop varieties, fertilization, irrigation, and pesticides causes soil degradation. As agriculture land becomes degraded, more forests are cut and converted for the needed agricultural production. This has led to a strong decrease of tropical rainforest area worldwide, especially in Southeast Asian countries (Gatto et al., 2015; Tarigan et al., 2015).

Indonesia is one of the tropical countries with highest deforestation rates, surpassing the rate in Brazil in 2012 (Hansen et al., 2013; Margono et al., 2014). Sumatra (Indonesia) lost more than half of its remaining natural rainforest between 1985 and 2007 due to deforestation and land-use intensification (Laumonier et al., 2010; Wilcove and Koh, 2010). Deforestation and agricultural intensification on the island is ongoing. Natural rainforests are converted to extensively managed agroforest (jungle rubber), then to intensively managed monoculture plantations (i.e., oil palm, rubber). These conversions are among the main drivers of deforestation aside from mining, timber and pulp industries (Guillaume et al., 2015; Villamor et al., 2014; Violita et al., 2015). However, extensive transformation of natural ecosystems to plantation leads to the decreased of soil fertility indicators and to subsequent soil degradation in Sumatra (Guillaume et al., 2016a, 2016b).

Land-use change significantly modifies the physical, chemical and biological soil properties, affects soil fertility, and increases erosion and compaction (Giessen et al., 2009; Matson et al., 1997; Moges et al., 2013). Phosphorus (P) is a key nutrient requiring attention in response to land-use change (Garcia-Montiel et al., 2000): it is the most limiting nutrient for plant productivity, especially in tropical regions (Dieter et al., 2010; Holford, 1997; Sanchez, 1976; Spohn et al., 2013; Vitousek, 1984). The highly weathered acidic soils and large quantities of sesquioxides adsorb and chemically fix P, leading to P limitations in tropical ecosystems (Bucher et al., 2001; Holford,

1997). Soil available P is mainly supplied by parent material, is recycled by decomposition of organic matter, and added by fertilizer inputs that enrich different P forms (i.e., available, moderately available, non-available inorganic and organic). When available P is depleted, replenishment from other P forms becomes important (Henriquez, 2002).

Land-use changes affect P availability for plant uptake either by increasing P losses or by transforming it to more recalcitrant pools. This leads to potentially significant effects on the distribution of P within chemically-defined pools, in turn determining availability and stability (Wright, 2009). Some studies on the partitioning of total soil P revealed effects of land-use change (Cassanova et al., 2002; Solomon et al., 2002). The fires – forest burning during plantation establishment – also impact soil P. They release P into the available pool, where it can be taken up by microorganisms, sorbed on the mineral matrix, leached or removed by runoff (Sanchez, 1976). At medium to high fire intensities (>300 °C), however, P mobilization is restricted and fixation increases. This is due to a heat-induced increase in mineral surface area, the production of Fe oxides free of organic matter and high affinity for P sorption (Ketterings et al., 2002). Short-term P fertilization is enhanced by ash of forest fires coupled with root decomposition of the original vegetation (Groppo et al., 2015). Nonetheless, fertility is not sustainable. Nutrient depletion occurs as plantations age (Numata et al., 2007; Townsend et al., 2002), reflecting nutrient removal with yield products. Furthermore, tremendous changes in plant biomass production and nutrient cycling due to vegetation conversions have a great negative or positive influence on soil properties and nutrient availability (Chen et al., 2003). The conversion of P from available to non-available (e.g., Al-P, Fe-P) and organic forms occurs in less than 50 years after land-use change, much faster than the thousands of years required under natural conditions (Garcia-Montiel et al., 2000). The conversion of forest to cropland decreased the P amount and increased the proportion of non-available P forms (Chacon and Dezzio, 2004). Accelerated soil erosion due to land-use change reduced organic matter by half or more (Zheng et al.,

2005), which is a source of organic substrate for nutrient release such as available P (Groppo et al., 2015; Pimentel et al., 1995).

Various approaches have been developed to study the forms, amount and dynamics of P cycling (Bowman and Cole, 1978; Chang and Jackson, 1957; Hedley et al., 1982; Tiessen and Moire, 1993). The sequential chemical fractionation developed by Hedley et al. (1982) has been widely used in recent decades to study soil P fractions and thus soil P dynamics (Chimdi et al., 2014). The chemical fractionation method evaluates the location and bonding type of P within the soil matrix (Guo et al., 2000; Yang and Post, 2011), and investigates the effects of land-use change on the distribution of P fractions. Hedley fractionation assumes that extractants of varying strength estimate inorganic phosphorus (Pi) and organic phosphorus (Po) fractions of different availability and chemical bindings (Guo et al., 2000; Hedley et al., 1982). The following fractions respond to extractants and are available: (i) H<sub>2</sub>O-Pi and NaHCO<sub>3</sub>-Pi, which are considered the most biologically and readily available Pi form. (ii) NaHCO<sub>3</sub>-Po, which is easily mineralizable and may contribute to the plant-available Pi. (iii) NaOH-P, which is associated with P and is strongly adsorbed via a covalent bond between phosphate oxygen and the aluminum (Al) and iron (Fe) in clays, which are involved in long-term P transformations. (iv) HCl-Pi, which is relatively insoluble P, often associated with Ca-P. HCl-Po has not been measured in most sequential P fractionation studies. It is reported that this fraction is Ca-bound hydrolysable Po.

Most studies in Sumatra (Indonesia) on the effects of land-use change and deforestation deal with soil carbon contents and stocks. This reflects the importance of low-carbon agriculture, climate change and general soil fertility issues. Nonetheless, only few studies focus on the effect of land-use change on soil P availability; no studies are available on P fractionation of various forms of inorganic and organic P. Our study is designed to assess the effects of land-use change on inorganic and organic P forms of different availability and on the P stocks in highly weathered tropical soils. We hypothesized that inorganic and organic P fractions of different availability will strongly decrease

after land-use change. Likewise, P stocks – the total of all P fractions – will also decrease.

### 2.2.3 Materials and Methods

#### 2.2.3.1 Study area and soil sampling

The study was carried out in Jambi Province in Sumatra, Indonesia. The climate is tropical humid with an average temperature of 27 °C and an average precipitation of 2200 mm yr<sup>-1</sup> and 112–259 mm month<sup>-1</sup> (Guillaume et al., 2015). Aside from tropical rainforest, the area had three dominating land-use types: (1) jungle rubber, (2) rubber plantation, and (3) oil palm plantation. Jungle rubber is an extensively-managed agroforest (minimum age of 16 yrs) in which rubber trees are planted in a partially logged forest. Tree species namely: *Alstonia* spp., *Artocarpus* spp., *Fabaceae* sp., *Macaranga* spp., *Porterandia* sp., and *Hevea* sp. are the most common tree species in the agroforest system. On the other hand, rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*) plantations were intensively managed monocultures of similar average age (14 yrs), ranging from 12 to 17 years (Guillaume et al., 2016a). Rubber and oil palm plantation received high NPK fertilization at a rate of 100-300 kg ha<sup>-1</sup> yr<sup>-1</sup> and 300-600 kg ha<sup>-1</sup> yr<sup>-1</sup>, respectively. Fertilization happens twice a year once in the rainy season (October to March) and once in dry season (April to September). Herbicides were also applied in both plantations every 6 months (Kotowska et al., 2015).

To assess the effects of land-use change, three replicate sites for each land-use type were selected within a distance of 16 km with an elevation varied between 50 and 100 m a.s.l. The soil was Acrisols with sandy loam texture. It is a highly weathered soil with strongly acidic soil pH ranged between 3.9 and 5.1. The base saturation ranged between 16 and 28 % and effective CEC ranged between 40 and 46 mmol<sub>c</sub> kg<sup>-1</sup> (Allen et al., 2015). At each site, samples were collected in one pit by horizons down a maximum depth of 1 m. Soils were air-dried and sieved at 2 mm. Plant debris and stones were removed. Soils were brought to the laboratory of the Department of Soil Science in Temperate Ecosystem in Göttingen University, Germany, for

further analysis. A detailed description of the study area and soil sampling are available in Guillaume et al. (2015). Further information on land-use history, management and soil characteristics can be found in Allen et al. (2015) and Kotowska et al. (2015).

### *2.2.3.2 Soil incubation and preparation*

Five grams of air-dried soil was placed in a glass bottle and incubated at field capacity at  $24 \pm 2^\circ\text{C}$  for 14 days prior to the sequential extraction in order to reach equilibrium after sampling, drying and sieving disturbances (Hedley et al., 1982). After the incubation, soils were stored at  $4^\circ\text{C}$  and equilibrated at room temperature overnight prior to P sequential fractionation analysis.

### *2.2.3.3 Phosphorus sequential fractionation*

The Hedley et al. (1982) sequential fractionation method as modified by Tiessen and Moir (1993) was used to fractionate soil P. This method uses a sequence of increasingly strong extractants that removed labile inorganic phosphorus (Pi) and organic phosphorus (Po) forms first, then stable P forms (Fig. A.1).

One gram of soil was placed into a 50 ml screw cap centrifuge plastic tube and sequentially extracted with the following extractants in sequential order: (i) 30 ml deionized water, which extracts fairly labile Pi (mobile P) that is directly exchangeable with the soil solution, (ii) 30 ml 0.5 M  $\text{NaHCO}_3$  at pH 8.5, which extracts relatively labile Pi and Po sorbed onto soil surfaces, plus a small amount of microbial P, (iii) 30 ml 0.1 M NaOH, which extracts amorphous and some crystalline Fe and Al phosphates, as well as P strongly bound by chemisorption to Fe and Al compounds; ultrasonication of soil for 2 min at 75 W in 0.1 M NaOH enabled extraction of P held at the internal surfaces of soil aggregates, (iv) 30 ml 1 M HCl, which extracts relatively insoluble Ca-P minerals including apatite, Al-P and Fe-P in more weathered soils. After every addition of extractant, samples were shaken continuously for 16 h using an end-to-end shaker, and the soil suspension was centrifuged at 3500 rpm for 15 min. Supernatant was filtered using Whatman no. 42 filter paper into small vials and stored at  $4^\circ\text{C}$  for phosphate determination. Finally,

soil residues were digested using concentrated  $\text{H}_2\text{SO}_4$  and 30%  $\text{H}_2\text{O}_2$  to extract more chemically stable Po forms and relatively insoluble Pi forms (Residual-P). We omitted the addition of an anion exchange membrane and chloroform from the original methodology.

### *2.2.3.4 Phosphate determinations*

Total P (TP) and Pi were measured directly from the extracts, while Po was calculated as the difference between the TP and Pi. TP was determined in 5 ml aliquots of each extract after ammonium-persulfate and  $\text{H}_2\text{SO}_4$  digestion to oxidize dissolved Po to Pi forms, and TP was measured as soluble reactive P (Environmental Protection Agency, 1971). Another 5 ml aliquot of each extract was acidified using 0.9 M  $\text{H}_2\text{SO}_4$  to precipitate organic matter and then used to measure Pi. The pH of final extracts for both TP and Pi was adjusted to pH 3 using dinitrophenol (2,4-DNP) as indicator. If yellow coloration formed after 2,4-DNP addition, diluted HCl was added drop by drop until the extracts turned colorless. Otherwise, if the extract remained colorless after 2,4-DNP addition, NaOH was added first until the colors changed to yellow and then dilute HCl until it became colorless again. Standards with increasing P concentrations were prepared. Phosphate concentrations for both standards and soil extracts were determined by molybdate colorimetry (Murphy and Riley, 1962) at 880 nm using a calibrated spectrophotometer (Specord 40). Po in the deionized water fractions and in the HCl-extractable fraction was not determined because preliminary investigations had shown values below the detection limit.

### *2.2.3.5 Bulk density and Carbon content determination*

Bulk density has been measured by another research group. It was done by core method using cylinders measuring two hundred and fifty  $\text{cm}^3$ . Cylinders were inserted horizontally at 5, 20, 40 and 75 cm depth from the side of the pit. Samples were air-dried and weighed. Carbon content was measured using an Elemental Analyser (Eurovector) after weighing 5 to 40 mg of grinded soil in tin capsules.



### 2.2.3.6 Data analysis

P fractions are expressed as the mean of the three field replicates and are presented in mg P kg<sup>-1</sup> of the fine earth (< 2 mm) fraction. All results are expressed based on oven-dried soil (105 °C, 24 h). In order to facilitate interpretations, P fractions were classified into three main groups (Tiessen et al., 1984): easily-available P, moderately-available P and non-available P. Easily-available P<sub>i</sub> is the sum of H<sub>2</sub>O-extractable and NaHCO<sub>3</sub>-extractable phosphate. Moderately-available P is the sum of P extracted from 0.5 M NaOH before and after sonification. Likewise, non-available P is the sum of P extracted by 1 M HCl and concentrated H<sub>2</sub>SO<sub>4</sub> and 30% H<sub>2</sub>O<sub>2</sub> during soil digestion. P stocks expressed in kg P ha<sup>-1</sup> were calculated for the soil depth intervals 0–20 and 0–60 cm using the following equation:

$$S = x \cdot \rho \cdot z \quad (1)$$

where *S* is the P stock for fixed depths and (*x*) is the soil P content at the designated depth (*z*), and *ρ* is the soil bulk density.

Differences in the P fractions, total P, P stocks and C/P ratio between agroforest and monoculture plantations were tested by one-way analysis of variance (ANOVA). Correlations between soil carbon content and P fractions were assessed by calculating linear regression. All analysis was performed using Statistix v8.1 statistical software.

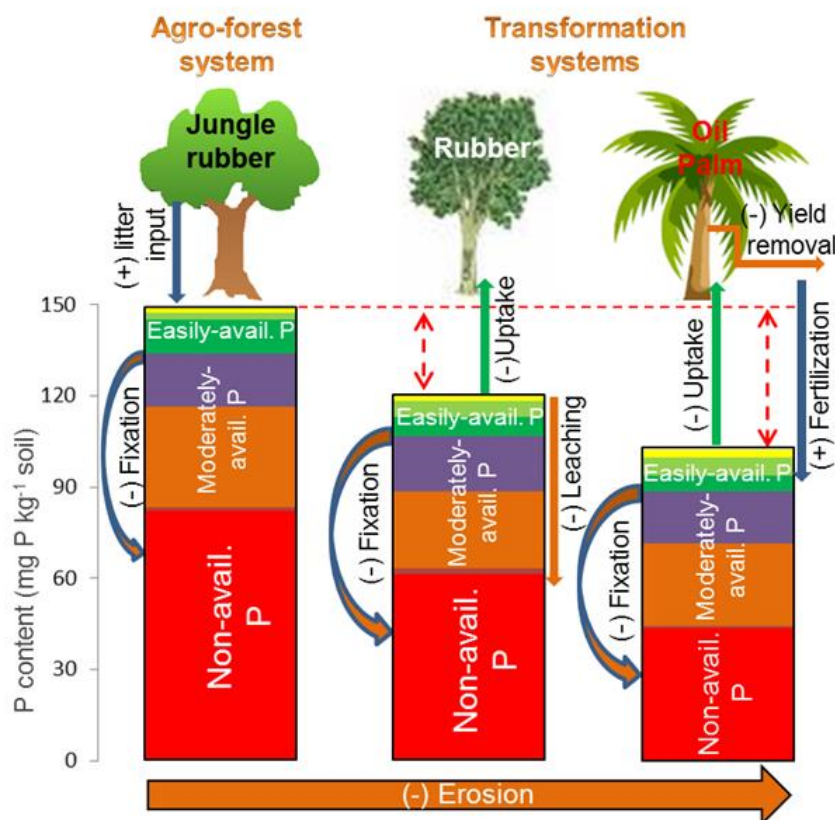
### 2.2.4 Results

#### 2.2.4.1 Concentrations of sequentially extracted phosphorus fractions

We recorded clear effects of land-use change of jungle rubber to oil palm and rubber plantations on inorganic and organic P fractions (Fig. 1). The content of easily-available, moderately-available and non-available P<sub>i</sub> and P<sub>o</sub> varied markedly among land-use types (Fig. 2). The change of land-use strongly decreased the content of P fractions in the topsoil (Ah horizon; 0–10 cm).

Easily-available P<sub>i</sub>, the sum of H<sub>2</sub>O-extractable and NaHCO<sub>3</sub>-P<sub>i</sub>, was the only P fraction that increased after land-use change (*P* < 0.05), indicating the effect of NPK fertilization applied twice a year. The oil palm plantation, which

received 2–3 times more NPK fertilizer than rubber, had more easily-available Pi ( $8.5 \pm 1.2 \text{ mg P kg}^{-1}$ ) in the top horizon than the rubber plantation ( $7.1 \pm 0.7 \text{ mg P kg}^{-1}$ ). The P increase due to fertilization was more notable in available Pi (labile-Pi) than in other P fractions. In contrast, jungle rubber, which did not

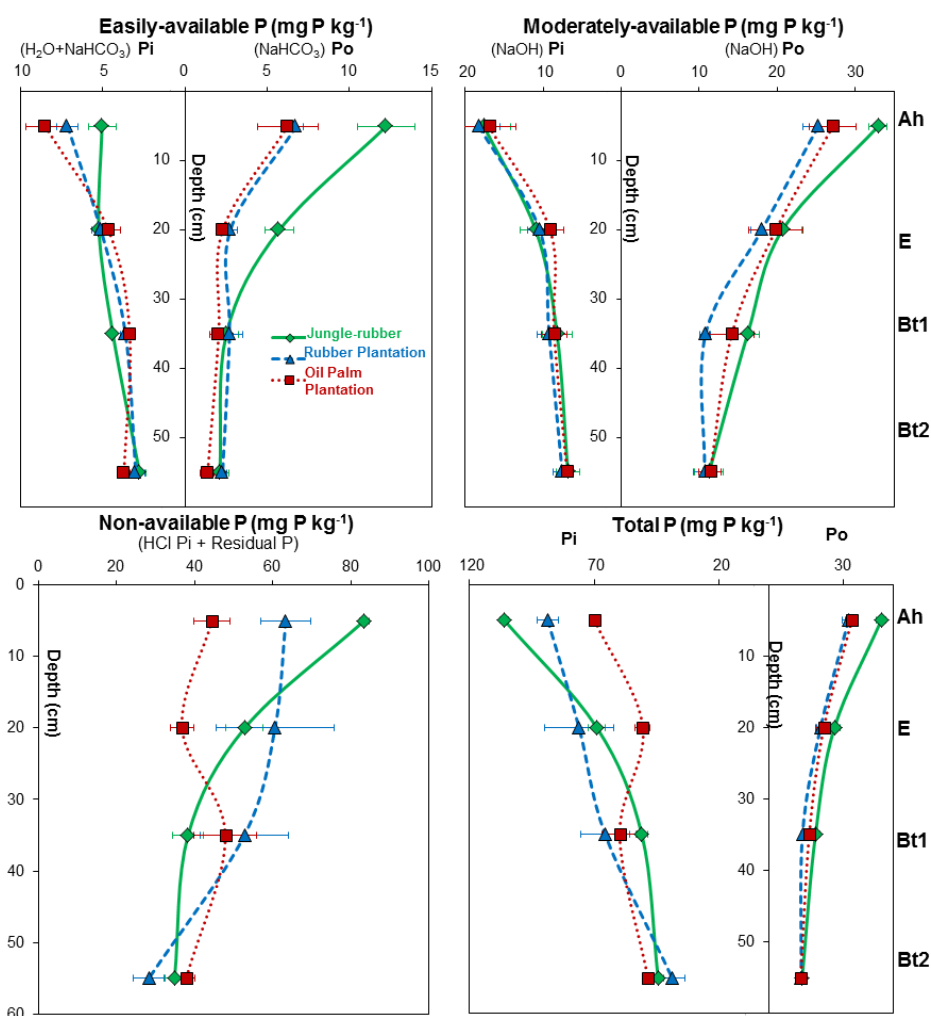


**Figure 1** Effects of land-use change on soil P. (+) increase P availability; (-) decrease P content and availability for plant uptake. Colors: yellow =  $\text{H}_2\text{O-Pi}$ ; yellow-green =  $\text{NaHCO}_3\text{-Pi}$ ; green =  $\text{NaHCO}_3\text{-Po}$ ; violet =  $\text{NaOH-Pi}$ ; light brown =  $\text{NaOH-Po}$ ; dark brown =  $\text{HCl-Pi}$ ; red = residual P. Red dashed line (----) shows P losses after land-use change.

receive any fertilization, had only  $5.0 \pm 0.9 \text{ mg P kg}^{-1}$ . However, easily-available Po ( $\text{NaHCO}_3$ -extractable Po), which is the most important P reserve, decreased ( $P < 0.05$ ) in plantations by almost 50% compared to the jungle rubber.

Moderately-available Pi ( $\text{NaOH-Pi}$ ) had the same content in all soils across all horizons. However, moderately-available Po decreased in the top horizon by 18% under oil palm and by 23% under rubber plantations compared to the jungle rubber. Differences between jungle rubber and the intensive plantations were significant ( $P < 0.05$ ) only in the Ah horizon (0–10 cm) but not at the

lower depths. Nonetheless, organic P fractions were almost twice as high as the inorganic P in all soils across depths. Non-available P in the topsoil (extracted by 1 M HCl and Residual-P) also decreased ( $P < 0.05$ ) by 47% under oil palm and by 24% under rubber plantations compared to jungle rubber, but there were no differences below 10 cm. Our results (Figs. 1 & 2) suggest that land-use change caused the redistribution of P forms.



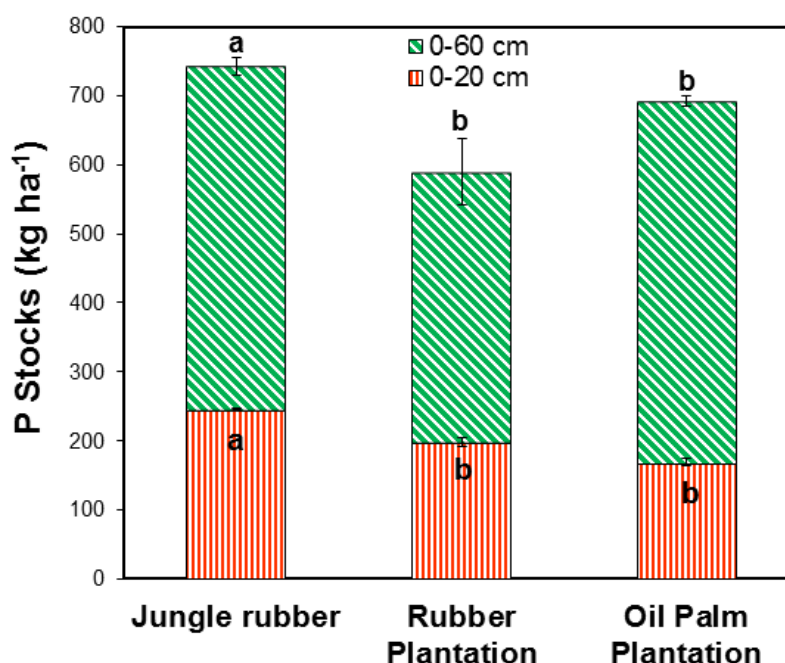
**Figure 2** Inorganic and organic soil phosphorus fractions (mg P kg<sup>-1</sup>) depending on land use. Values represent means  $\pm$  SE (n=3).

#### 2.2.4.2 Total phosphorus content and phosphorus stocks

Most of the total P in soils of each land-use type was inorganic. It ranged between 39 and 106 mg P kg<sup>-1</sup>, which represented between 68 and 83% of the total P (Fig. 2). Jungle rubber had more total Pi in the Ah horizon than in intensive monoculture plantations (i.e. oil palm and rubber). Total Po was also

higher ( $P < 0.05$ ) in the Ah horizon in jungle rubber than in the intensive monoculture plantations.

The soil P stock decreased after conversion of jungle rubber to an intensively managed oil palm plantation at the 0–20 cm depth by about 31%, and slightly increased by 5% at the 0–60 cm depth (Fig. 3). Soil P stocks also gradually decreased in the rubber plantation by 20% from 0 to 60 cm depth. P stocks were lost in both plantation systems ( $P < 0.05$ ) compared to jungle rubber at 0–20 cm depth. Consequently, land-use change strongly decreased P stocks, especially in the topsoil.

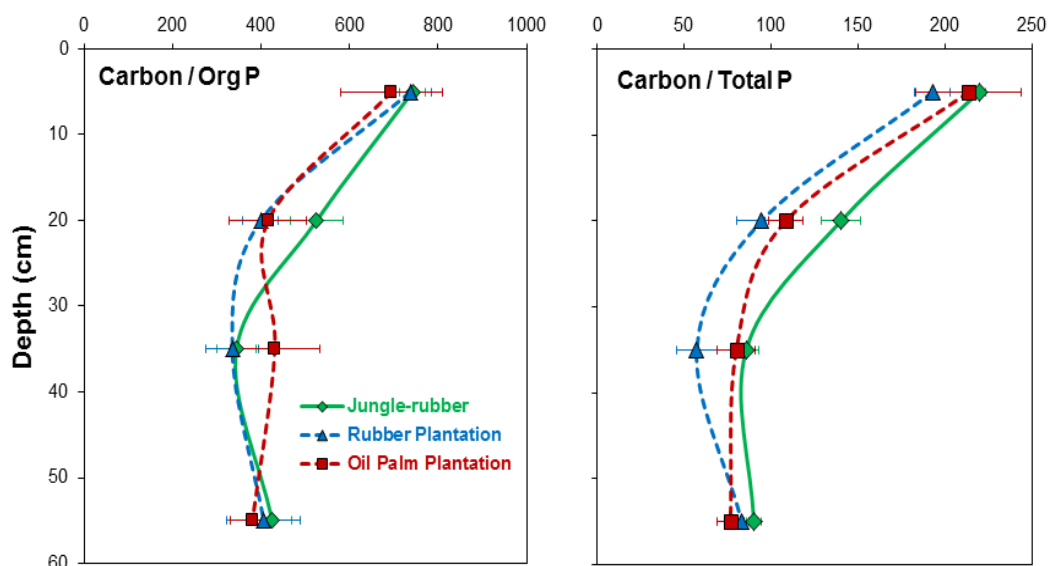


**Figure 3** Soil phosphorus stocks ( $\text{kg P ha}^{-1}$ ) at the 0-20 and 0-60 cm soil depth layer depending on land use. Values represent means  $\pm$  SE ( $n=3$ ). Means followed by different letters within the same depth differ significantly ( $t$ -test at  $P < 0.05$ ).

#### 2.2.4.3 Carbon to phosphorus ratio

The carbon:organic P (C:Po) ratio calculated using total organic P content ranged between  $695 \pm 342$  and  $742 \pm 88$  in surface soils, being greatest in soils with the least total soil Po (Fig. 4). The lowest C:Po ratio ( $335 \pm 181$ ) was measured at 35 cm depth in the rubber plantation. Moreover, the ratio declined from the surface down to 35 cm and stabilized in the subsoil, but not

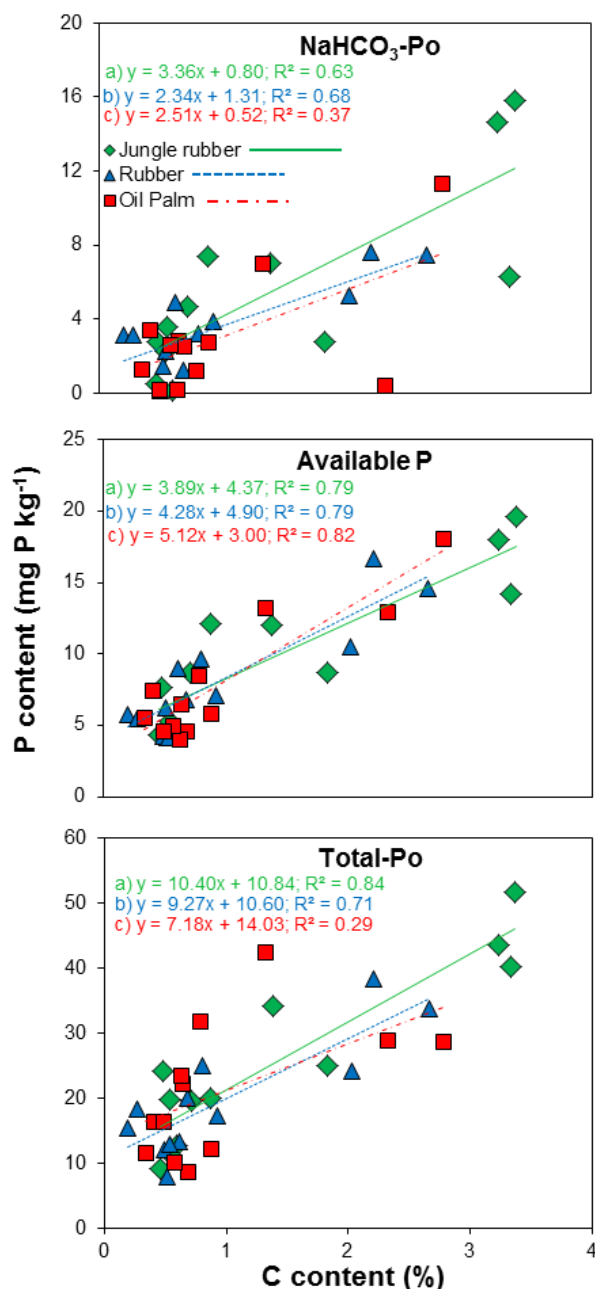
under oil palm. Likewise, the C:TP ratio calculated using the total P (sum of all P fractions) had the same pattern as the C:Po ratio in all land-use types. Both ratios declined from the surface down to 35 cm depth and tended to stabilize in the lower depth. The C:TP ratio ranged between  $57 \pm 34$  and  $220 \pm 12$ , being highest in jungle rubber across all soil depths.



**Figure 4** Ratio of (left) soil carbon to organic phosphorus, and (right) soil carbon to total phosphorus depending on land use. Values represent means  $\pm$  SE ( $n=3$ ).

#### 2.2.4.4 Correlation between phosphorus fractions and soil carbon

Total Po was correlated with soil C ( $R^2 = 0.29\text{--}0.84$ ;  $P < 0.001$ ) (Fig. 5). The correlation was strongest in jungle rubber ( $R^2 = 0.84$ ) compared to monoculture plantations. Easily-available P fractions ( $H_2O$ ,  $NaHCO_3$  Pi and Po) were also strongly correlated ( $R^2 = 0.79\text{--}0.82$ ;  $P < 0.001$ ) with soil C in all land-use types. Easily-available P was dominated by  $NaHCO_3$ -extractable Po, and had the strongest correlation to soil C ( $R^2 = 0.37\text{--}0.68$ ;  $P < 0.001$ ) compared to  $H_2O$ -P and  $NaHCO_3$ -Pi in all land-use types. Easily-available P correlated well with C in jungle rubber and rubber compared to oil palm plantation. This suggests that SOM contributes to the P fertility in the soil.



**Figure 5** Relationship between soil carbon content and (top) NaHCO<sub>3</sub>-Po, (middle) available-P (i.e., H<sub>2</sub>O-extractable P, NaHCO<sub>3</sub>-extractable inorganic and organic P), (bottom) total Po depending on land use. Very close correlation between all land uses for NaHCO<sub>3</sub>-Po, available-P and total-Po to soil carbon reflects P losses by erosion.

## 2.2.5 Discussion

### 2.2.5.1 Effect of land-use change on the forms and distribution of soil P

Inorganic and organic P forms of different availability are influenced by anthropogenic, biotic and abiotic processes that either increase or decrease the soil P content (Fig. 1 & Table 1). The higher concentrations of easily-

available Pi (i.e., H<sub>2</sub>O-Pi and NaHCO<sub>3</sub>-Pi) (Fig. 2) that we found in intensive plantations are explained by the adsorption of P from fertilizers in the surface layer (Henriquez, 2002; Neufeldt et al., 2000). The increase in P content due to fertilization is more notable in available Pi (labile Pi) than in other P fractions (e.g., Neufeldt et al., 2000). At the same time, the depletion of easily-mineralizable Po (NaHCO<sub>3</sub>-Po) could be attributed to the decrease of litterfall accumulated in the plantation. The aboveground litterfall of rubber (3.84 Mg ha<sup>-1</sup> year<sup>-1</sup>) and oil palm (6.23 Mg ha<sup>-1</sup> year<sup>-1</sup>) plantation was lower than jungle rubber litterfall (7.66 Mg ha<sup>-1</sup> year<sup>-1</sup>) (Kotowska et al., 2016).

**Table 1** Direct effects of abiotic, biotic and anthropogenic processes on P fractions in soil.

Processes/Mechanism		Easily-available		Moderately-available		Non-available
		Pi	Po	Pi	Po	Pi + Po
Anthropogenic	P fertilization (Inorganic & Organic)	↑↑↑	↑↑	↑	↑	↑
Biotic	Litter input	↑	↑↑		↑	
	Mineralization	↑↑	↓↓↓		↓	
	Plant uptake	↓↓↓	↓↓↓	↓↓	↓	
	Immobilization in microorganism	↓↓↓	↓↓↓		↑↑	↑↑
Abiotic	Erosion	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
	Leaching	↓↓	↓	↓		
	Fixation/Adsorption	↓↓↓	↓↓	↑↑	↑	↑↑
	Desorption	↑↑		↓↓		↓

↑↑↑ strong increase; ↑↑ moderately increase; ↑ slight increase  
 ↓↓↓ strong decrease; ↓↓ strong decrease; ↓ slight decrease

The high accumulation of litterfall in jungle rubber resulted to high easily-mineralizable Po. Soil without tillage for 20 years builds up a considerable amount of Po from accumulated SOM (Magid, 1993). Furthermore, the vertical distribution of Po is related to the distribution of SOM in the soil profile (Sarapatka, 2003). Vertical trends of easily-available P that declined from the surface to the lower depths is explained by a process termed “nutrient pumping” (Kautz et al., 2013). This means that trees are able to absorb nutrients from lower soil horizons and then redistribute them to the surface

soil through litterfall and throughfall (Farley and Kelly, 2004). Easily-available P content was dominated by  $\text{NaHCO}_3\text{-Po}$  and strongly correlated with soil C (Fig. 5). This highlighted the potential importance of SOM in maintaining fertility in tropical ecosystems (Tiessen et al., 1994).

P extracted by 0.1 M NaOH before and after ultrasonification is associated with humic compounds and mostly adsorbed to Al and Fe oxides in acidic soils (Hedley et al., 1982; Schoenau et al., 1989). This fraction is considered to be moderately-available P that involves long-term soil P transformation (Hedley et al., 1982; Tiessen et al., 1984) and acts as a buffer for labile  $\text{P}_i$  in highly weathered soils (Guo et al., 2000). Reduced moderately-available  $\text{P}_o$  (Fig. 2) in plantations is also due to less litter input than in jungle rubber. As litter inputs dropped, the soil capacity to retain P in the form of NaOH-extractable  $\text{P}_o$  diminished (Zamuner et al., 2008). In contrast, P fixation by Al and Fe is repressed, reducing available P in the long term (Dieter et al., 2010; Groppo et al., 2015). Nonetheless,  $\text{P}_o$  fractions were almost twice as more as the  $\text{P}_i$  fractions in all soils across all depths. This highlights the importance of  $\text{P}_o$  as P reserves that involve long-term soil P transformation and in P cycling when soil  $\text{P}_i$  reserves are limited (Buehler et al., 2002).

The importance of plant non-available P forms has been frequently reported (Neutfeldt et al., 2000; Reddy et al., 1999; Sharpley, 1985; Tiessen et al., 1984; Zheng et al., 2002). Depending on soil type and management, non-available P forms could be mobilized and become available for plant uptake, depending on P form, undergoing desorption, weathering, and mineralization processes. Land-use change decreases non-available P (Fig. 2), which suggests lower P reserves compared with agroforest systems.

### *2.2.5.2 Phosphorus status and long-term sustainability*

Soil total P represents the long-term potential of the P supply, whereas easily-available  $\text{P}_i$  represents the short-term bioavailability. Moderately available and non-available P (Figs. 1 & 2) decreased after land-use change, which contributes to the decrease of total P in intensive monoculture plantations. Land-use change, however, increases only easily-available  $\text{P}_i$  ( $\text{H}_2\text{O-P}_i$  and



NaHCO<sub>3</sub>-Pi) due to fertilization, which merely maintains short-term soil fertility. Nonetheless, it is not sustainable in the long run due to the depletion of soil P reserve fractions (i.e., NaHCO<sub>3</sub>-Po, moderately-available P and non-available P). The higher correlation (Fig. 5) between total Po and soil C was mainly attributable to NaHCO<sub>3</sub>-extractable Po versus NaOH-extractable Po. This further indicates that litter mineralization significantly contributes to the available P content (Fig. 2).

The decreased soil P stocks at 0–20 cm depth (Fig. 3) suggests that the addition of NPK fertilizer did not compensate for the amount of P lost after conversion of extensively managed (jungle rubber) to intensive plantations. The strong soil P loss after intensification is closely linked to soil erosion. This interpretation is supported by Guillaume et al. (2015), who estimated strong soil erosion under rubber and oil palm plantations. Accelerated soil erosion due to land-use change reduced SOM by half or more (Zheng et al., 2005), which is a source of available P when mineralized. About 50% of total P is contained in SOM (Pimentel et al., 1995). The soil removed by erosion is 1.3 to 5 times richer in OM than the remaining soil (Matson 1997; Pimentel et al., 1995). A combination of decreasing litter inputs (when plantation crops are replaced rainforest) and increasing soil erosion leads to a decrease of SOM and P in the long run (Chimdi et al., 2014). Another contribution to the P depletion in plantation soils is nutrient export with the yield (Kotowska et al., 2015). This pertains especially to the reproductive parts of oil palm trees that are removed from the ecosystem and not returned back to the soil (Violita et al., 2015). Indeed, three years of litter removal resulted in a marked reduction of Po in the surface soil (Vincent et al., 2010). At the same time, the slight increase (5%) of P stocks under oil palm at 0–60 cm depth might be attributed to leaching. Some of the fertilizers applied at the soil surface were leached or removed by erosion. Such leached P fertilizers accumulated at lower depths ( $\geq 35$  cm depth) where Al and Fe accumulation were high. This slightly boosted (5%) P stocks of oil palm in the lower depth compared with jungle rubber. The P fixation, however, decreased the availability of P for plant uptake.

### 2.2.5.3 *The importance of organic phosphorus for P availability*

In tropical ecosystems, the turnover of OM is a very important source of Po that is widely considered to be fundamental in maintaining the P supply (Condrón and Tiessen, 2005). Available P in highly weathered soils is generally low and depends on Po mineralization (Tiessen et al., 1984). Po in the available pool (NaHCO<sub>3</sub>-Po) is very important because it increases the apparent P availability (Johnson et al., 2003). Likewise, Po, which occurred in moderately-available (NaOH-Po), is as important as NaHCO<sub>3</sub>-Po because it contributes to P reserves. Both Po fractions decrease after land-use change (Figs. 1 & 2), mainly due to soil erosion and yield export.

The C:Po ratios can be used to assess the nutrient status of a site. An adequate supply of available phosphate for plant growth prevents Po mineralization by phosphatases. This results in an accumulation of soil Po and a reduced C:Po ratio (Dieter et al., 2010; Zhao et al., 2008). If available P is insufficient for plant growth, phosphatase synthesis increases (Spohn and Kuzyakov, 2013). This, in turn, enhances Po mineralization compared to C mineralization and increases the C:Po ratio (Dieter et al., 2010; McGill and Cole, 1981). If soil has a high available P content, then the C:Po ratio is <100, whereas the ratio in soils with insufficient available P is >200 (Dieter et al., 2010; Smeck, 1985). Based on the above considerations, the declining C:Po ratio of plantation soils (Fig. 4) indicates the effect of available P from the added NPK fertilizer. Nonetheless, the C:Po ratio in each land-use type (>200) confirms that the soils in the study area are P limited. The low available P in all land-use types is due to the inherently low-P status of the parent material and erosion losses (Henriquez, 2002; Moges et al., 2013).

### 2.2.6 **Conclusions**

The forms and distribution of P in various land-use systems is determined by anthropogenic, biotic and abiotic processes. The proportion of Pi was higher than Po in soil under each land-use type. Pi was mainly composed of chemically more stable (NaOH-Pi) and relatively insoluble P forms (i.e., HCl-Pi and Residual-P) rather than available-Pi in all land-use types. The effects of the management after land-use change of jungle rubber to oil palm and

rubber plantations on Pi and Po fractions were underlined. Short-term high NPK fertilization increased easily-available Pi in intensive monoculture plantations. At the same time, easily-mineralizable Po decreased due to depletion of SOM. Moderately-available P and non-available P also decreased after land-use change of jungle rubber to oil palm and rubber plantations. Land-use change leads to an overall reduction of P stocks due to a strong decrease of SOM caused by erosion and yield export of rubber or of oil palm seeds. Fertilization did not compensate for the P losses. Fertilization increases solely the available Pi in the topsoil. This maintains or increases fertility only over the short term, but decreases it over the long term by depleting P reserves (e.g., moderate or non-available P). The positive strong correlation between soil C and Po and the C:Po ratio indicates that mineralization from SOM strongly contributes to the available P content. This highlights the potential of SOM in maintaining P reserves. This calls for proper plantation management practices to stop SOM losses.

### 2.2.7 Acknowledgement

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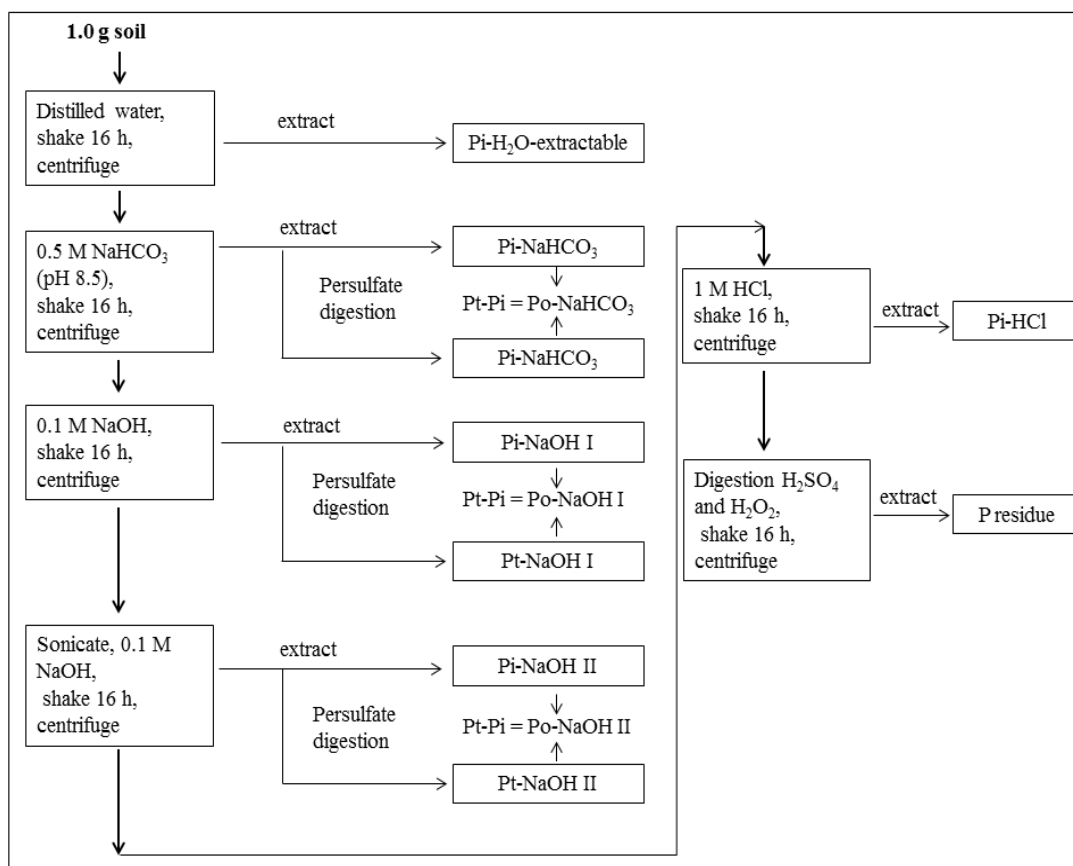
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## 2.2.9 Supporting Information



**Appendix 1** Diagram of Hedley P sequential fractionation scheme as modified by Tiessen and Moir (1993).

### 2.3 Study 3: Effects of flooding on phosphorus and iron mobilization in highly weathered soils under different land-use types: Short-term effects and mechanisms

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#### 2.3.1 Abstract

The strong affinity of phosphorus (P) to iron (Fe) oxides and hydroxides in highly weathered tropical soils limits P availability and therefore plant productivity in tropics. In flooded soils, however, P fixed by Fe oxides and hydroxides can be released into more available forms because of Fe<sup>3+</sup> reduction to Fe<sup>2+</sup>. These P dynamics in flooded soils are well documented for rice paddies. Such effects are much less studied in other land-use types influenced by seasonal flooding, especially in the tropics during heavy monsoon rains. The aim of this study was to investigate the P mobilization during flooding leading to anaerobic conditions in topsoil and subsoil depending on land-use type. Samples were collected in highly weathered Acrisols from four replicate sites under natural rainforest, jungle rubber, rubber and oil palm plantations in Sumatra, Indonesia. Topsoil and subsoil were taken to ensure a wide range of soil organic matter (SOM) and P contents. Soils were incubated under anaerobic, flooded conditions at 30 ± 1 °C for 60 days. Our results confirmed the hypothesis that soil flooding mobilizes P and increases P availability. Two distinct and opposite periods were observed during the flooding. During the first three weeks of flooding, the dissolved P (DP) concentration peaked, simultaneously with the peak of dissolved Fe<sup>2+</sup> (DFe<sup>2+</sup>) and dissolved organic carbon (DOC). After three weeks, P availability in soils decreased, although Fe-P (P<sub>NaOH</sub>) and available P (P<sub>NaHCO<sub>3</sub></sub>) did not reach the initial, pre-flooding levels. The impacts of flooding on P and Fe forms was strong in

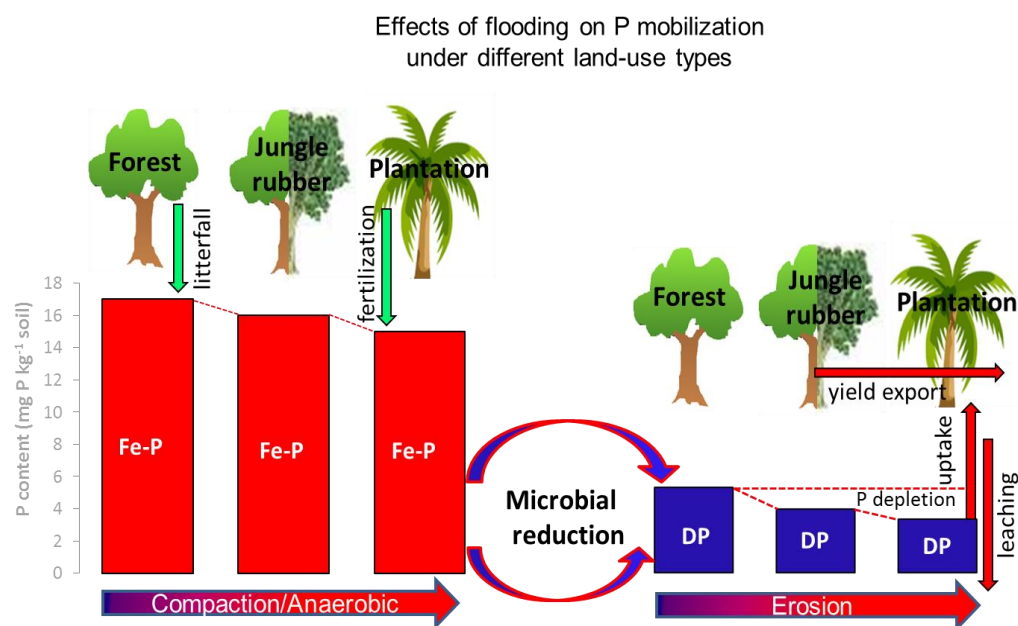


the topsoil, where P dissolution and availability were generally higher under forest and, to a lesser extent, under jungle rubber. A positive correlation between DOC and  $DFe^{2+}$  ( $R^2 = 0.42$ ) in topsoil indicates that the intensity of microbially-mediated  $Fe^{3+}$  reduction is limited by the amount of available carbon (C) as an energy source for microorganisms and as electron donor. Microbial mineralization of organic P from SOM also increases P availability, and this process requires available C. This interpretation was supported by the strong correlation ( $R^2 = 0.58$ ) between available P and DOC, as well as between DP and DOC ( $R^2 = 0.56$ ) in topsoil. The increasing pH in topsoil and subsoil after flooding of all land-use types may also influence the P release over time. In summary, the increase of available P and DP during flooding is due to three main mechanisms: (1) P release via the microbially-mediated reductive dissolution of  $Fe^{3+}$  oxides; (2) P release during SOM mineralization and (3) solubility of Fe phosphate due to increasing pH. These mechanisms are relevant not only in riparian areas, where flooding occurs, but also in soils waterlogged after regular heavy rainfalls during the wet season. Therefore, we speculate that the P turnover is faster in compacted soils under plantations because of regular changes of oxic and anoxic conditions. Consequently, more P is pumped by the vegetation and then removed from plantations due to yield export.

**Keywords:** Phosphorus mobilization; Flooding; Anaerobic conditions; Iron reduction; Tropical agroforest; Phosphorus pools

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### Graphical Abstract



### 2.3.2 Introduction

Phosphorus (P) in most soils and especially in the tropics is limited for plant uptake due to its immobilization on iron (Fe) and aluminum (Al) oxides (Dieter et al., 2010; Holford 1997; Maranguit et al., 2017). These bonds are very stable. Nonetheless, P sorption with Fe oxides might be reversible under anaerobic conditions, e.g., after flooding (Parker and Beck, 2003; Ponnampereuma, 1972; Rakotoson et al., 2015, 2016). Large seasonal fluctuations in rainfall, typical of tropical forested ecosystems, can change available P and are highest directly after the onset of the wet season (Wood et al., 2015; Wood and Lawrence, 2008). Flooding increases the available P content by 1.4–60 mg P kg<sup>-1</sup> compared with aerobic soils (Rakotoson et al., 2014). This is indicated by the increase of extractable P such as NaHCO<sub>3</sub>-extractable P (Verma and Tripathi, 1982; Zhang et al., 2003).

Once flooded, soils rapidly become anaerobic, resulting in a decline in the redox potential (Eh) (Ponnampereuma, 1972). The microbial community structure shifts to microbes capable of anaerobic respiration (Unger et al., 2009). Microorganisms utilize alternative electron acceptors such as NO<sub>3</sub><sup>-</sup>, Mn<sup>4+</sup>, Fe<sup>3+</sup> and SO<sub>4</sub><sup>2-</sup> to maintain their metabolism (Loeb et al., 2008; Unger et al., 2009). They use the electron acceptor that yields the highest energy or that is most readily available. In highly weathered acidic soils, Fe<sup>3+</sup> hydroxides are very abundant. Thus, microorganisms such as *Geobacter sulfurreducens* (Sánchez-Alcalá et al., 2011) will use Fe<sup>3+</sup> as the terminal electron acceptor (Weber et al., 2006). Hence, Fe<sup>3+</sup> will be reduced to Fe<sup>2+</sup>, releasing substantial quantities of associated P (Amarawansa et al., 2015; Loeb et al., 2008; Ponnampereuma, 1972). Therefore, the P concentration in the soil solution will increase together with soluble Fe<sup>2+</sup> (Kirk, 2004; Quintero et al., 2007). This mechanism, leading to an increase of P and Fe solubility under anaerobic respiration, is known as microbially-mediated reductive dissolution of Fe<sup>3+</sup> oxides.

The amount of P released into the soil solution depends on: soil characteristics involved in reduction processes: 1) abundance of Fe oxides and their crystallinity; 2) soil organic matter (SOM) content and its microbial

availability as electron donors (Quintero et al., 2007; Scalenghe et al., 2002) ; 3) total P content and its forms (Amarawansa et al., 2015) and 4) soil pH neutralization as a result of soil flooding, which increases the P availability by increasing the solubility of Fe- and Al-P compounds in acid soils (Chacon et al., 2005; Zhang et al., 2003). Additionally, SOM is used as a source of carbon (C) and energy by microorganisms to fuel and stimulate the microbially-mediated reductive dissolution of Fe<sup>3+</sup> minerals (Rakotoson et al., 2015; Scalenghe et al., 2002; Zhang et al., 1994). Hence, we hypothesize that P released by Fe<sup>3+</sup> reduction is stimulated in soil with high labile C availability. Furthermore, we hypothesize that P release is influenced by land-use changes because of their impacts on SOM and P contents.

Land-use conversion is the predominant global change in this century, driven by the high demand for food, fiber and other products (Geissen et al., 2009; Guillaume et al., 2015). In the tropics, for example in Indonesia, agricultural intensification is ongoing mainly for rubber and oil palm at the expense of primary and secondary forest (Gibbs et al., 2010; Guillaume et al., 2016). Forest conversion in general, strongly changes soil physical, chemical and biological properties (Geissen et al., 2009; Moges et al., 2013) as well as ecosystem functioning (Barnes et al., 2014) especially after conversion to rubber and oil palm monoculture plantation. Indeed, almost 70% SOC in the topsoil of oil palm and rubber plantations has been lost compared to rainforest in Indonesia (Guillaume et al., 2015). P forms changes from easily available to non-available forms due to P fixation by Fe and Al oxides. Organic P which is considered as reserve pool buffering available inorganic P becomes depleted and total P decreases after conversion of forest to oil palm and rubber plantations (Maranguit et al., 2017). Moreover, rubber and especially oil palm plantations suffer from soil compaction, resulting in higher bulk density and less water infiltration (Guillaume et al., 2016; Merten et al., 2016). Plantation soils are therefore quickly waterlogged by regular heavy rainfalls. This results in a series of biogeochemical changes that profoundly influence P status and availability. No studies are available on transformed systems in Sumatra, Indonesia, in particular studies focusing on the mobilization of P forms (Fe-

bound P) that are normally retained by well-drained soils when these become partly waterlogged or flooded by regular heavy rainfalls during the wet season. Moreover, most of the literature on Fe dynamics and P availability after soil flooding pertains to rice paddies (e.g., Ponnampereuma, 1972; Rakotoson et al., 2015; Zhang et al., 2003).

In this study, we investigated the effects of flooding on the P and Fe dynamics in topsoil (0–10 cm) and subsoil (20–30 cm) horizons of Acrisol under forest, agro-forest (jungle rubber) and monoculture plantations of rubber and oil palm on Sumatra, Indonesia. The study was designed to assess changes in Fe and P solubility and mobility under flooded anaerobic incubation. We hypothesized that under flooding: (1)  $\text{Fe}^{3+}$  will be reduced to  $\text{Fe}^{2+}$ , thereby liberating P adsorbed on  $\text{Fe}^{3+}$ , as indicated by P in the solution and  $\text{NaHCO}_3$ -Extractable P in the soil; (2) the content of P bound to Fe oxides, which is measured by  $\text{NaOH}$ -Extractable P, will decrease; and (3)  $\text{Fe}^{2+}$  and P in the soil solution will increase and will be higher in the topsoil under forest and jungle rubber, which have a higher SOM content than soils under monoculture plantations.

### 2.3.3 Materials and Methods

#### 2.3.3.1 Study site and soil sampling

The soil samples were collected in Jambi Province in Sumatra, Indonesia. The climate is tropical humid with an average temperature of 27 °C and an average precipitation of 2200 mm year<sup>-1</sup> and 112–259 mm month<sup>-1</sup> (Guillaume et al., 2015). Experiments were carried out in (1) tropical rainforest and three land-use types dominating in the study region: (2) jungle rubber, (3) rubber plantations and (4) oil palm plantations. Jungle rubber is an extensively-managed agroforest in which rubber trees are planted in a partially logged forest. Rubber and oil palm plantations were intensively managed monocultures. A detailed description of the study site is available in Guillaume et al., 2015.

To assess the effects of soil flooding on P mobilization, four replicate sites for each land-use type were selected. The soil was Acrisols with sandy loam texture. At each site, samples were collected in the topsoil (Ah horizon; 0–10

cm) and in the subsoil (20–30 cm) by digging a pit. These topsoil and subsoil samples were taken to ensure a wide range of soil properties with regard to SOM and P content (Table 1). Soil samples were air-dried and sieved at 2 mm. Plant debris and stones, if present, were removed.

**Table 1.** Characteristics of the soils use (means  $\pm$  SE; n = 4).

Land-use	Soil Parameter				
	pH	C	Avail-Pi <sup>a</sup>	Fe-Pi <sup>b</sup>	HCl-Fe(II)
	1:2.5	(%)	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )
Topsoil (0-10 cm)					
Forest	3.8	6.77 $\pm$ 0.64	10.0 $\pm$ 0.10	15.9 $\pm$ 0.41	2.01 $\pm$ 0.25
Jungle rubber	4.2	4.03 $\pm$ 0.57	5.03 $\pm$ 0.85	17.6 $\pm$ 0.49	5.23 $\pm$ 0.19
Rubber	4.4	2.56 $\pm$ 0.32	7.13 $\pm$ 0.65	18.2 $\pm$ 0.72	5.02 $\pm$ 0.26
Oil palm	4.3	2.05 $\pm$ 0.47	8.49 $\pm$ 1.16	16.9 $\pm$ 0.51	3.68 $\pm$ 0.20
Subsoil (20-30 cm)					
Forest	4.5	0.55 $\pm$ 0.18	5.73 $\pm$ 0.76	5.49 $\pm$ 0.38	2.02 $\pm$ 0.51
Jungle rubber	4.4	0.74 $\pm$ 0.30	4.37 $\pm$ 0.15	8.22 $\pm$ 0.91	3.23 $\pm$ 0.19
Rubber	4.3	0.87 $\pm$ 0.19	3.61 $\pm$ 0.29	9.23 $\pm$ 0.53	2.02 $\pm$ 0.65
Oil palm	4.3	0.62 $\pm$ 0.18	3.35 $\pm$ 0.36	8.59 $\pm$ 0.62	1.68 $\pm$ 0.70

<sup>a</sup> Available inorganic P that was extracted using 0.5 M NaHCO<sub>3</sub> at pH 8.5

<sup>b</sup> Fe-bound inorganic P that was extracted using 0.1 M NaOH

### 2.3.3.2 Soil flooding and incubation

2.5 g of soil sample were filled into a 12 ml glass tube (Labco Exetainer). Six milliliters of purified distilled water were added in each tube and air was driven out by purging N<sub>2</sub> gas. The suspension was then covered with a rubber stopper to prevent O<sub>2</sub> diffusion, evaporation losses and to ensure anaerobic conditions. Four field replicates of each land-use type and depth for each determination were incubated in the dark at 30  $\pm$  1 ° C. Directly after water addition (1 h) and after 7, 14, 21, 28, 45, and 60 days of continuous soil flooding, samples were shaken and pH was determined. Then, samples were filtered using a syringe filter with 0.45  $\mu$ m pore size (Labsolute, Germany). The extracts for dissolved P (DP) and dissolved Fe<sup>2+</sup> (DFe<sup>2+</sup>) determination were acidified immediately with 1 ml of 0.1 M HCl per 2 ml of solution to prevent oxidation. The remaining extracts were analyzed for dissolved organic

C (DOC) (see section 2.3). The samples were further analyzed for available-P and Fe-P (see section 2.4).

### 2.3.3.3 Determination of DOC, DP and $D\text{Fe}^{2+}$ in the soil solution

Dissolved C was measured using a 'multi N/C 2100' (Analytik jena) analyzer. Dissolved C corresponds to DOC because no carbonates were present in the soil (Guillaume et al., 2015).

DP was determined by the malachite green (MG) colorimetric method (D'Angelo et al., 2001; Yevdokimov et al., 2016). Briefly, 150  $\mu\text{l}$  of extracts was mixed with 30  $\mu\text{l}$  of the first reagent (ammonium molybdate tetrahydrate and sulfuric acid) in disposable sterile 96-well polystyrene microtiter plates (Puregrade, Germany). These plates were shaken for 10 min on an orbital shaker at low speed ( $<90 \text{ rev min}^{-1}$ ). Thereafter, 30  $\mu\text{l}$  of the second reagent, which was a mixture of MG carbinol hydrochloride and polyvinyl alcohol (Sigma Aldrich Co), was added. The plate was shaken for an additional 20 min. After shaking, samples were exposed to 40  $^{\circ}\text{C}$  for 40 min. To decrease variability between the replicates, microplates were left overnight at room temperature. Thereafter, absorbance was read using a spectrophotometer (TECAN; Infinite M200 pro) with 630 nm wavelength. Standards were also prepared in triplicate and treated the same way as the samples.

$\text{Fe}^{2+}$  was determined by ferrozine assay (Stookey, 1970). In principle, the  $\text{Fe}^{2+}$  ions in the solution form complexes with the ferrozine and form a magenta-colored solution. In brief, 1 ml of extract was mixed with 100  $\mu\text{l}$  ferrozine solution and then with 50  $\mu\text{l}$  buffer solution (ammonium acetate adjusted to pH 9.5 with ammonium hydroxide) in a 24-well polystyrene microplate (Puregrade, Germany). Then, the microplate was read in a spectrophotometer (TECAN; Infinite M200 pro) with 562 nm wavelength after color developed fully, normally after 5 min.

### 2.3.3.4 Determination of available-P and Fe-P in the soil

Soil samples after filtration were measured for  $\text{NaHCO}_3$ - and  $\text{NaOH}$ -extractable P. In this experiment,  $\text{NaHCO}_3$ -extractable P and  $\text{NaOH}$ -

extractable P refer to available-P and Fe-bound P (Fe-P), respectively. The available-P and Fe-P was assessed following the Hedley P fractionation method (Hedley et al., 1982; Maranguit et al., 2017). Briefly, 1 g of wet soil sample was placed into a 50 ml screw cap centrifuge plastic tube. Samples were sequentially extracted with 30 ml of 0.5 M NaHCO<sub>3</sub> (pH 8.5) to extract available-P and, thereafter, with 0.1 M NaOH to extract P bound with Fe. Samples were shaken for 16 h in an orbital shaker, centrifuged at 3500 rpm for 10 min and then filtered. Phosphorus was determined in all extracts and standards by the malachite green colorimetric method (D'Angelo et al., 2001; Yevdokimov et al., 2016) (*as mentioned in section 2.3*).

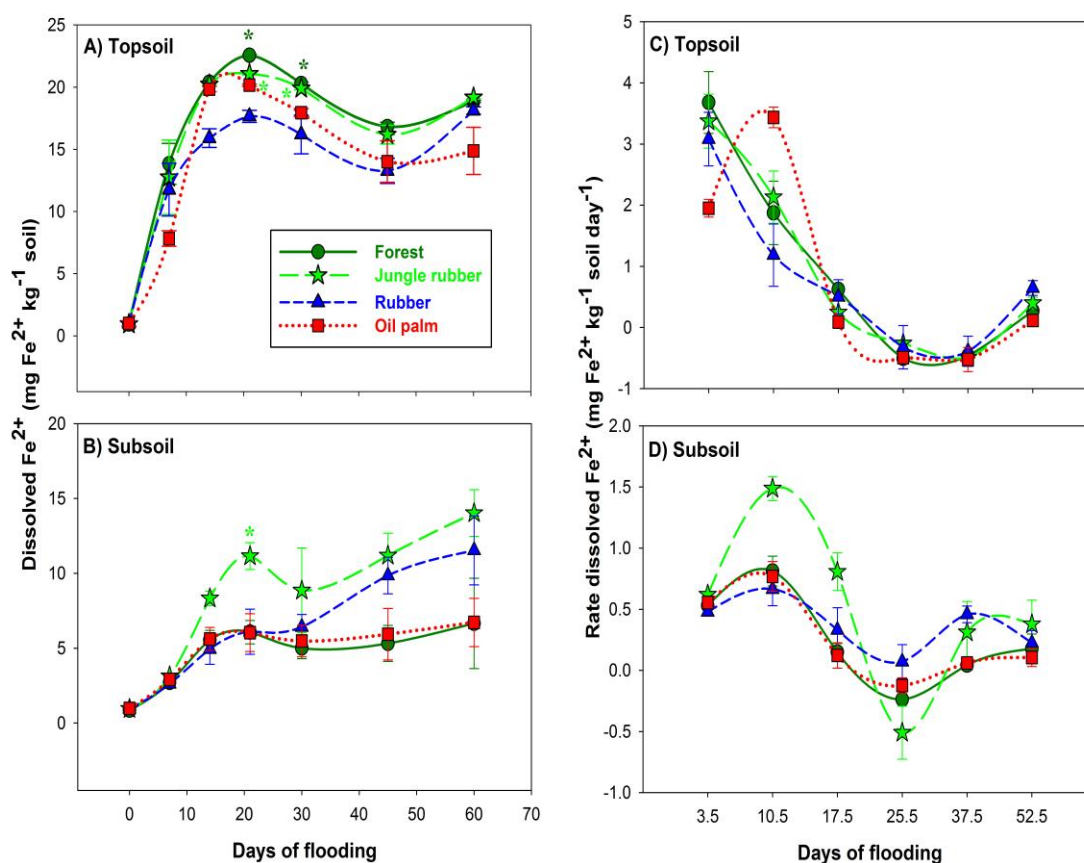
### 2.3.3.5 Data analysis

Results of all parameters were expressed as the mean of four field replicate samples and were presented as mg kg<sup>-1</sup> or mg g<sup>-1</sup> of the dry fine earth (< 2 mm) fraction. Normality and homogeneity of variance were checked using Shapiro-Wilk's *W* test and Levene tests, respectively. Differences of the soil parameter between land-use types for each sampling period were tested using one-way analysis of variance (ANOVA). To check the influence of land-use on the changes of soil parameters between two time points, two-way repeated measures ANOVA were tested with (4) land-use and (2) time as the factors. For the changes between two sampling periods of one land-use, single sample *t*-tests (testing means against zero) on the rate of DFe<sup>2+</sup>, DP and DOC were conducted. A *t*-test result not significantly different to 0 means no changes between the two sampling period. Relationships between P availability indices (i.e., avail-P, Fe-P, DP) and associated soil parameters (i.e., DOC, DFe<sup>2+</sup>) were evaluated using Multiple Linear Regression. Relationships between two parameters were tested on the values recorded after 21 days of soil flooding; the time when Fe<sup>2+</sup> reduction and P release peaked. Significance was accepted at a probability level of  $p \leq 0.05$ . All statistical analyses were carried out using STATISTICA 12 (StatSoft Inc., USA)

## 2.3.4 Results

### 2.3.4.1 $Fe^{3+}$ reduction and $Fe^{2+}$ mobilization

The  $Fe^{2+}$  concentration in the soil solution was used as an index of  $Fe^{3+}$  reduction (Stookey, 1970). An increasing concentration of  $DFe^{2+}$  in the soil solution (Fig. 1) indicates the intensity of reduction. Soil flooding reduces  $Fe^{3+}$



**Figure 1** Effects of soil flooding on  $DFe^{2+}$  under forest, jungle rubber, rubber and oil palm plantations. We compared the  $DFe^{2+}$  of the topsoil (A) and subsoil (B). The computed rates of  $DFe^{2+}$  production in the topsoil (C) and subsoil (D) are also shown.  $DFe^{2+}$  on day 0 indicates the initial content determined after 1 h of soil flooding. Values represent means  $\pm$  SE ( $n=4$ ). Asterisks in (A) and (B) show significant differences (one-way ANOVA;  $p < 0.05$ ) to rubber and oil palm soils at a given sampling time.

to  $Fe^{2+}$  under anaerobic conditions already within a few days. The reduction of  $Fe^{3+}$  to  $Fe^{2+}$  had already started one week after soil flooding, as highlighted by an increased ( $p < 0.001$ )  $DFe^{2+}$  concentration after 7 days (Fig. 1).

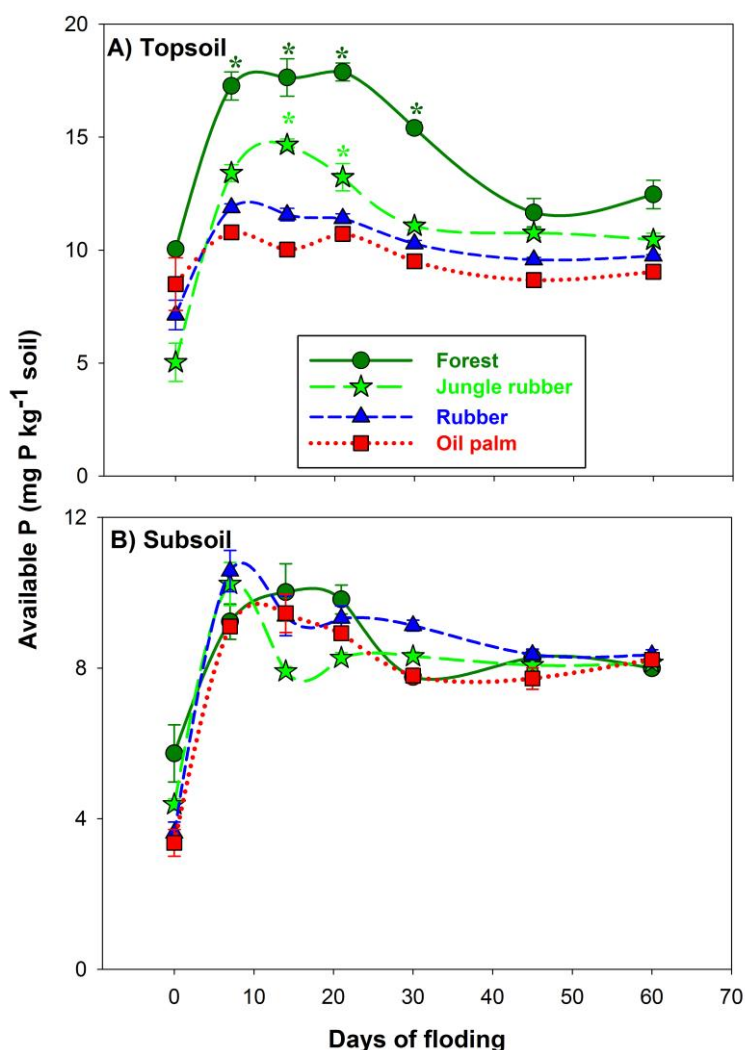
$DFe^{2+}$  in topsoil reached its maximum after 21 days of flooding in all land-use types. The highest  $DFe^{2+}$  peak ( $p < 0.05$ ) was in forest ( $22.6 \pm 0.1$  mg  $Fe^{2+}$  kg $^{-1}$



<sup>1</sup>), followed by jungle rubber ( $21.1 \pm 0.1 \text{ mg Fe}^{2+} \text{ kg}^{-1}$ ), oil palm ( $20.2 \pm 0.1 \text{ mg Fe}^{2+} \text{ kg}^{-1}$ ) and rubber ( $17.6 \pm 0.5 \text{ mg Fe}^{2+} \text{ kg}^{-1}$ ) (Fig. 1). The highest  $\text{Fe}^{2+}$  production rate was about 4 days after soil flooding in all land-use types except for oil palm, in which highest production occurred at about 11 days. Note, however, that the  $\text{DFe}^{2+}$  production in all land-use types diminished over time until the last flooding day. In fact, the rate was negative between days 26 and 38 of flooding ( $t$ -test;  $n = 4$ ;  $p < 0.05$ ), indicating re-precipitation of  $\text{DFe}^{2+}$ . In contrast, subsoils reached their maximum accumulation of  $\text{DFe}^{2+}$  in the last week (60 days) of soil flooding in all land-use types, showing that reduction processes in the subsoil were slower. The highest production rate of  $\text{DFe}^{2+}$  in subsoil was recorded about 11 days after flooding (Fig. 1). Jungle rubber showed higher rates ( $p < 0.05$ ) than other land-use types throughout the soil flooding period. In all land-use types, topsoil had higher ( $p < 0.01$ )  $\text{DFe}^{2+}$  than subsoil throughout the flooding period (Fig. 1). This shows that the  $\text{DFe}^{2+}$  production rate is much faster in the topsoil, indicating the importance of SOM and microorganisms in Fe reduction.

### 2.3.4.2 Phosphorus mobilization and availability

Soil flooding increases P availability ( $p < 0.05$ ), as shown by the increase of  $\text{NaHCO}_3$ - extractable P in both topsoil and subsoil in all land-use types after one week of flooding (Fig. 2). The available P content of the forest and jungle rubber topsoil started to increase after the first week of soil flooding and continued until it peaked after 21 days, i.e. at the same time when the  $\text{DFe}^{2+}$  (Fig. 1) in the soil solution was at its maximum. The maximum increase of available P in forest (21 days =  $17.9 \text{ mg P kg}^{-1} \pm 1.6$ ) and jungle rubber (14 days =  $14.7 \text{ mg P kg}^{-1} \pm 1.0$ ) was about 50–70% of the initial content before flooding (Fig. 2). Forest and jungle rubber had higher ( $p < 0.05$ ) available P compared to monoculture plantations throughout the flooding period. In contrast, the topsoil of monoculture plantations had a more or less stable available P content after an increase ( $p < 0.05$ ) in the first week of flooding

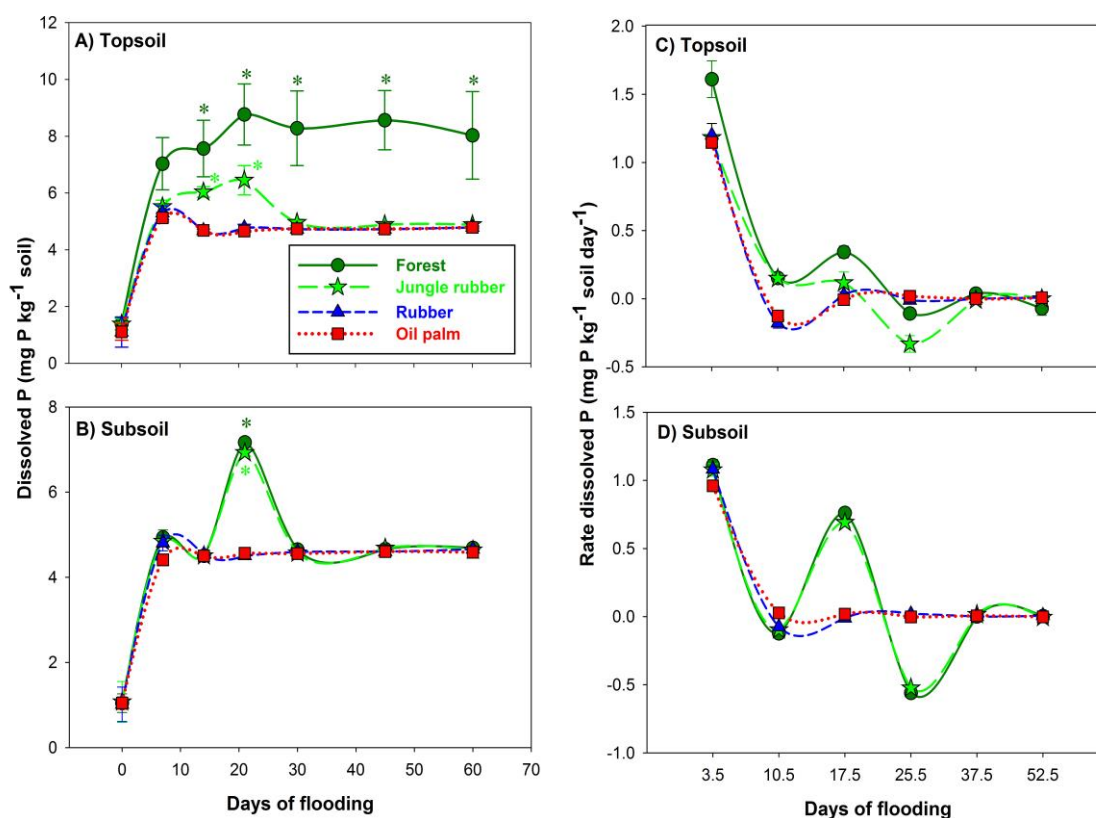


**Figure 2** Effects of soil flooding on the available P ( $P_{NaHCO_3}$ ) of topsoil (A) and subsoil (B) under forest, jungle rubber, rubber and oil palm plantations. Available P on day 0 indicates the initial content before soil flooding. Values represent means  $\pm$  SE ( $n=4$ ). Asterisks show significant differences (one-way ANOVA;  $p < 0.05$ ) to rubber and oil palm soils at a given sampling time.

(Fig. 2). The same pattern was observed in the subsoil of all land-use types; the subsoil showed an increase in available P only after one week and then stabilized until the last day of flooding.

The effect of land-use type on DP production was similar to that on  $DFe^{2+}$  production (forest > jungle rubber > oil palm > rubber) throughout the flooding period. DP release started one week after soil flooding (Fig. 3), at the same time when  $Fe^{3+}$  reduction and  $DFe^{2+}$  production also started (Fig. 1). In all land-use types, the DP rates in the topsoil were highest 4 days after flooding (Fig. 3). The land-use influence on DP production was evident in the early

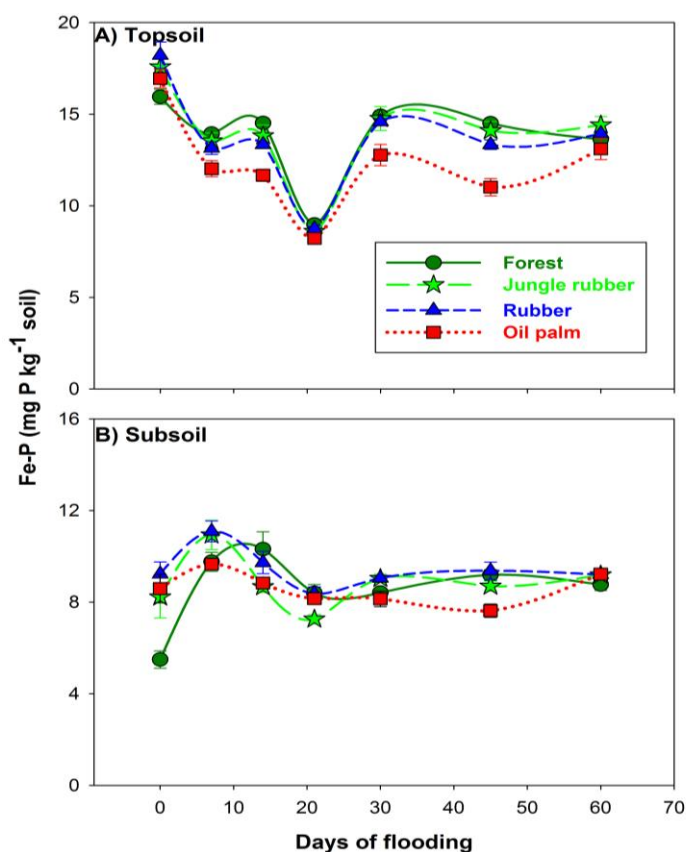
stage of flooding (Table A.1). The topsoil of forest showed the highest ( $p < 0.05$ ) DP concentration ( $1.61 \text{ mg P kg}^{-1} \pm 0.13$ ) of all land-use types. DP production ceased 26 days after soil flooding, after which constant values were recorded (Fig. 3). A different trend was observed in the monoculture plantation in both topsoil and subsoil. DP increased only after 7 days of soil flooding with a peak of  $6.0 \text{ mg P kg}^{-1}$  and  $5.0 \text{ mg P kg}^{-1}$  in topsoil and subsoil, respectively, and it remained stable thereafter (Fig. 3).



**Figure 3** Effects of soil flooding on DP under forest, jungle rubber, rubber and oil palm plantations. We compared the DP of the topsoil (A) and subsoil (B). The computed rates of DP production in the topsoil (C) and subsoil (D) are also shown. DP on day 0 indicates the initial content determined after 1 h of soil flooding. Values represent means  $\pm$  SE ( $n=4$ ). Asterisks in (A) and (B) show significant differences (one-way ANOVA;  $p < 0.05$ ) to rubber and oil palm soils at a given sampling time.

#### 2.3.4.3 Changes of Fe-bound phosphorus (Fe-P)

The Fe-P (Fig. 4), which was determined by NaOH-extractable P, clearly decreased after flooding. The maximum decrease of about 50% ( $p < 0.05$ ) was observed in the third week, at about the time when maximum P was liberated. The highest P released ( $p < 0.05$ ) in the topsoil versus subsoil in all

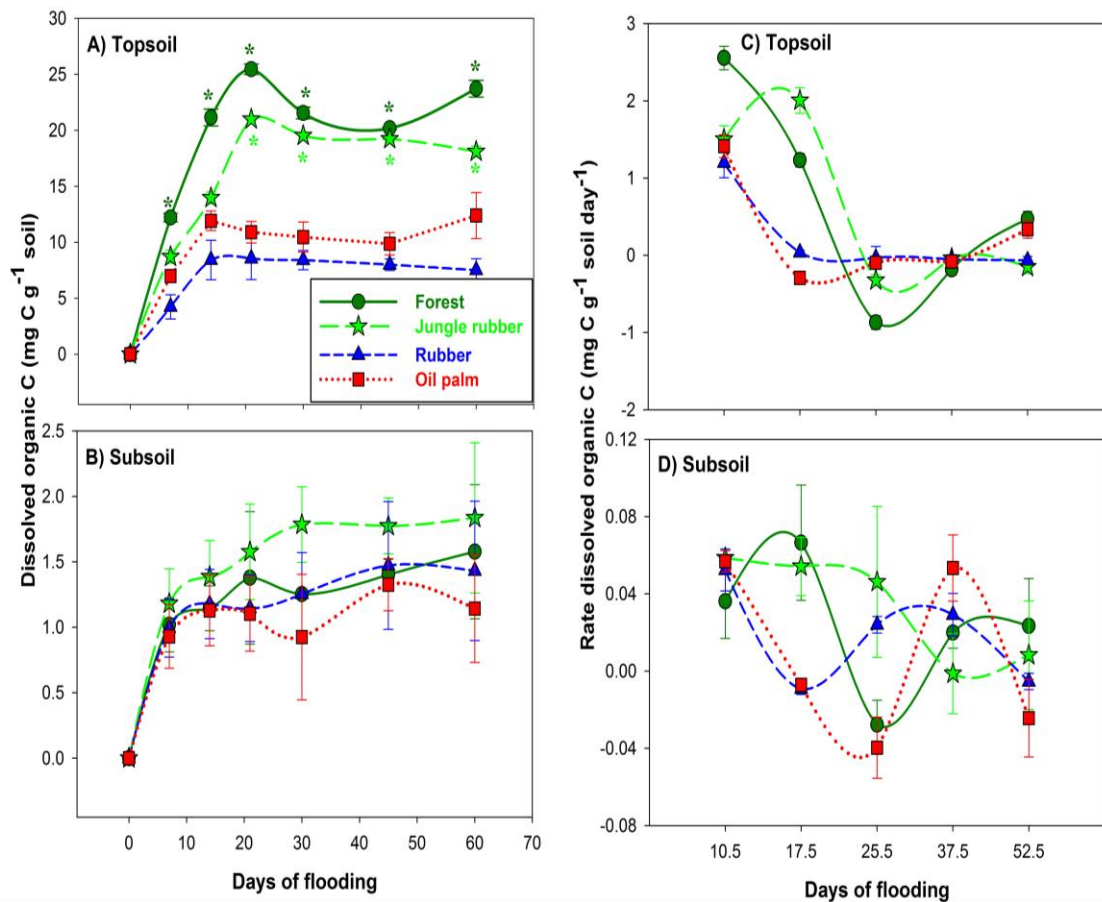


**Figure 4** Effects of soil flooding on the Fe-bound P ( $P_{NaOH}$ ) of topsoil (A) and subsoil (B) under forest, jungle rubber, rubber and oil palm plantations. Fe-P on day 0 indicates the initial content before soil flooding. Values represent means  $\pm$  SE ( $n=4$ ).

land-use types (Fig. 3) corresponds to the Fe-P content (Fig. 4) in the initial state of soil flooding: less Fe-P was present ( $p < 0.05$ ) in the subsoil than in the corresponding topsoil, and thus the P release in subsoil was less ( $p < 0.05$ ) than in topsoil throughout the flooding period. Nevertheless, a sharp decline ( $p < 0.05$ ) in available P and DP after one month caused the Fe-P content to increase towards its initial level.

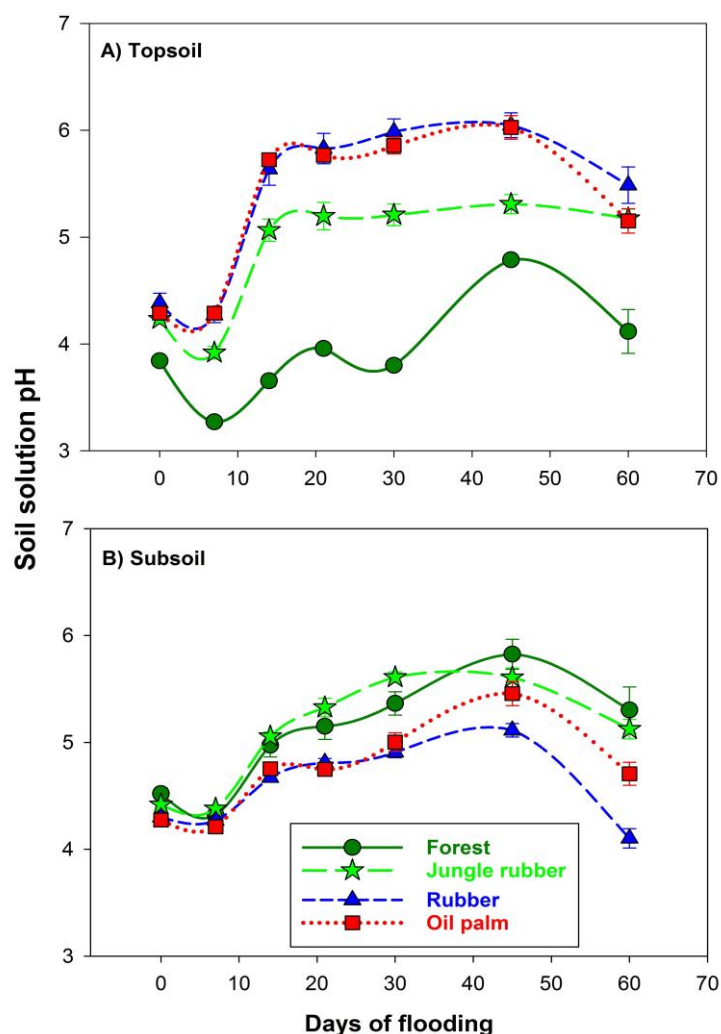
#### 2.3.4.4 Changes of DOC and soil solution pH

Soil flooding also influenced the DOC concentration (Fig. 5). DOC ( $p < 0.05$ ) was highest in the topsoil of both forest and jungle rubber during the early stage of flooding. This reflects the high SOM content under forest and jungle rubber compared to plantation soils (Table 1). This also explains the 10 times higher DOC ( $p < 0.05$ ) in the topsoil versus subsoil in all land-use types throughout the flooding period.



**Figure 5** Effects of soil flooding on DOC under forest, jungle rubber, rubber and oil palm plantations. We compared the DOC of the topsoil (A) and subsoil (B). The computed rates of DOC production in the topsoil (C) and subsoil (D) are also shown. Values represent means  $\pm$  SE ( $n=4$ ). Asterisks in (A) and (B) show significant differences (one-way ANOVA;  $p < 0.05$ ) to rubber and oil palm soils at a given sampling time.

The soil solution pH declined in the first flooding week (Fig. 6). Afterwards, it marginally increased (average of 1 unit) in all soils until 45 days and then started to decline again. The trend was similar in both topsoil and subsoil of all land-use types. These results suggested that, in flooded acidic soils, pH will increase and stabilize at a new level after an initial decline. Consequently, this pH change appears to be one of the prerequisites for the solubilization and release of adsorbed P.



**Figure 6** Effects of soil flooding on soil pH of topsoil (A) and subsoil (B) under forest, jungle rubber, rubber and oil palm plantations. pH on day 0 indicates initial value before soil flooding. Values represent means  $\pm$  SE (n=4).

### 2.3.5 Discussion

Our data confirmed the results of the previous investigations that soil flooding mobilizes P and increases P availability (e.g., Ponnampereuma, 1972). Interestingly, we found two distinct and opposite phases occurred upon flooding which were not found in the previous investigations. In the first phase during the first three weeks (with maximal intensity in the first week),  $DFe^{2+}$ , DP and DOC accumulated in the soil solution (Figs. 1, 3 and 5, respectively). In parallel, the P availability in soil increased, as indicated by a decrease in Fe-P (Fig. 4) and an increase in available-P (Fig. 2). This shows that the

impacts of flooding are fast and not limited to P release; rather, soil P forms were also modified to more available fractions. Microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  oxides was important during the first three weeks of flooding, as indicated by the significant increase of  $\text{DFe}^{2+}$  during this period. The similar trend of  $\text{Fe}^{2+}$  and P release in the soil solution (Figs. 1 and 3, respectively) and the decrease of Fe-P (Fig. 4) confirmed that the reduction and dissolution of Fe(hydr)oxides releases their adsorbed or occluded phosphate (Rakotoson et al., 2015). Our results are in line with the findings of Ponnampertuma (1985), who reported that soil flooding resulted in a decline of Fe-P content and an increase of DP as a result of  $\text{Fe}^{3+}$  reduction, hydrolysis and dissolution of Fe-P in flooded soil. The increase of the available P fraction in soil suggests that part of the Fe-P affected by iron oxide reduction was not directly released in the soil solution but remained adsorbed on soil particles in a form available for plants, thus reducing potential P losses through leaching.

The Fe-P pool is the main source from which P is released during reduction (Loeb et al., 2008; Zhang et al., 2003). This reduction is influenced by flooding duration. Despite the sharp increase of  $\text{DFe}^{2+}$  (Fig. 1) during the first three weeks, a significant DP (Fig. 3) release occurred mainly in the first week (except under forest, where a slight but significant accumulation lasted two additional weeks). Accordingly,  $\text{Fe}^{3+}$  oxides with high Fe-P content were exhausted after a week of flooding, and the subsequent microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  oxides occurred on  $\text{Fe}^{3+}$  oxides with lower Fe-P content. It is also possible that a larger fraction of Fe-P affected by the reduction of  $\text{Fe}^{3+}$  oxides in a later stage of flooding was not released as DP but contributed to the more available soil P pool.

Land-use types influenced the impacts of flooding on P and Fe forms mainly in the topsoil, where P dissolution and availability were generally higher under forest and, to a lesser extent, under jungle rubber. Faster solubility in topsoil and especially under forest is connected with a higher C content there (Guillaume et al., 2015), leading to faster microbial consumption of  $\text{O}_2$  and consequently faster and more intensive  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  reduction. Land-use type effects were not related to initial differences in P or Fe forms because the

differences in P and Fe forms were generally much bigger after three weeks of flooding than the initial differences. This indicates that process intensities during flooding were affected by land-use type. The different P concentration between land-use types (Fig. 3) released after soil flooding is explained by the amount of SOM influencing microorganisms. SOM is used as a source of C and energy by the microorganisms and stimulates the microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  minerals (Rakotoson et al., 2015; Scalenghe et al., 2002; Zhang et al., 1994). The presence of high native SOM in forest and jungle topsoil drove  $\text{Fe}^{3+}$  reductions and increased the reduction intensity. Indeed, we recorded a positive relationship ( $R^2 = 0.42$ ;  $p < 0.01$ ; Table 2) between  $\text{DFe}^{2+}$  and DOC of the topsoil during early flooding. Furthermore, the high DOC in forest and jungle rubber (Fig. 5) highlighted an efficient  $\text{Fe}^{3+}$  reduction and high release of sorbed P in both forest and jungle rubber topsoil compared to rubber and oil palm soil. Nonetheless, it remains unclear whether DOC was a source of C for microorganisms, thereby enhancing  $\text{Fe}^{3+}$  reduction, or whether it was a by-product of SOM decomposition during  $\text{Fe}^{3+}$  reduction, thereby being an indicator of the microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  minerals. The direct effect of the low SOM in subsoil in all land-use types resulted to a smaller release of P and Fe compared to topsoil. In conclusion, the extent of microbially-mediated  $\text{Fe}^{3+}$  reduction was apparently limited by C availability, as observed in other studies (Loeb et al., 2008; Rakotoson et al., 2014, 2015). Accordingly, land-use types affected P mobilization and availability under flooding conditions mostly because forest conversion to plantations strongly decreased SOM.

Part of the P release may originate from mineralization of P bound in SOM, especially during lengthier flooding. Microbial decomposition of SOM to scavenge organic C as an energy source for  $\text{Fe}^{3+}$  reduction was accompanied by the mineralization of organic P. Several studies have hypothesized that organic P mineralization from SOM is mainly driven by the microorganisms' need for C as their energy (e.g., Achat et al., 2012; Spohn and Kuzyakov, 2013). This hypothesis was verified in the topsoil of all those land-use types with high SOM concentrations, strengthening the land-use effects on P



release. This was supported by a strong correlation between available P and DOC ( $R^2 = 0.58$ ;  $p < 0.001$ ; Table 2) and between DP and DOC ( $R^2 = 0.56$ ;  $p < 0.001$ ; Table 2) in the topsoil of all land-use types during early flooding. Nonetheless, the doubling of the DOC concentration in forest between the second and third week of flooding was not followed by the same increase in DP, indicating that DP accumulation was also triggered by factors beyond an accumulation of organic P bound to DOC or released from SOM.

**Table 2.** Correlation coefficient ( $R^2$ ) between concentrations of P forms and associated soil attributes after 21 days (maximum P release) of soil flooding.

	DOC	DFe <sup>2+</sup>
<b>Avail-P</b>		
Surface	0.58 <sup>***</sup>	0.53 <sup>**</sup>
Subsoil	0.29 <sup>ns</sup>	0.08 <sup>ns</sup>
<b>DP</b>		
Surface	0.56 <sup>***</sup>	0.38 <sup>*</sup>
Subsoil	0.18 <sup>ns</sup>	0.13 <sup>ns</sup>
<b>DOC</b>		
Surface	----	0.42 <sup>**</sup>
Subsoil	----	0.10 <sup>ns</sup>

\*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; ns=not significant; ---- not applicable

The increasing soil solution pH in topsoil and subsoil across all land-use types may also have influenced the release of P over time. Most reduction reactions consume  $H^+$ , increasing the pH in acid soils; e.g.,  $Fe(OH)_3 + 3H^+ + e \rightarrow Fe^{2+} + H_2O$  (Narteh and Sahrawat, 1999; Ponnampereuma, 1972). The neutralization of soil pH as a result of reduction processes during flooding increases the solubility of Fe- and Al-P compounds and the desorption of surface P in acid soils (Ponnampereuma, 1972). According to (Kashem and Singh, 2001), soil pH will approach neutrality (6.5 to 7.5) regardless of the initial value before flooding. In our experiments, soil pH increased with flooding time. However, the first week of flooding caused a pH to decline (Fig. 6). This was due to the accumulation of  $CO_2$  produced by respiration of aerobic bacteria (Ponnampereuma, 1972) and the influence of continuous production of organic acids because forest and jungle soils contain abundant SOM. The lower pH of forest and jungle topsoil throughout the incubation period was due to the buffering effect of SOM that were abundant in both soils compared to plantation soils. Nonetheless, the influence of soil pH on P

release was likely low because pH increased only after one week, when most P was already released.

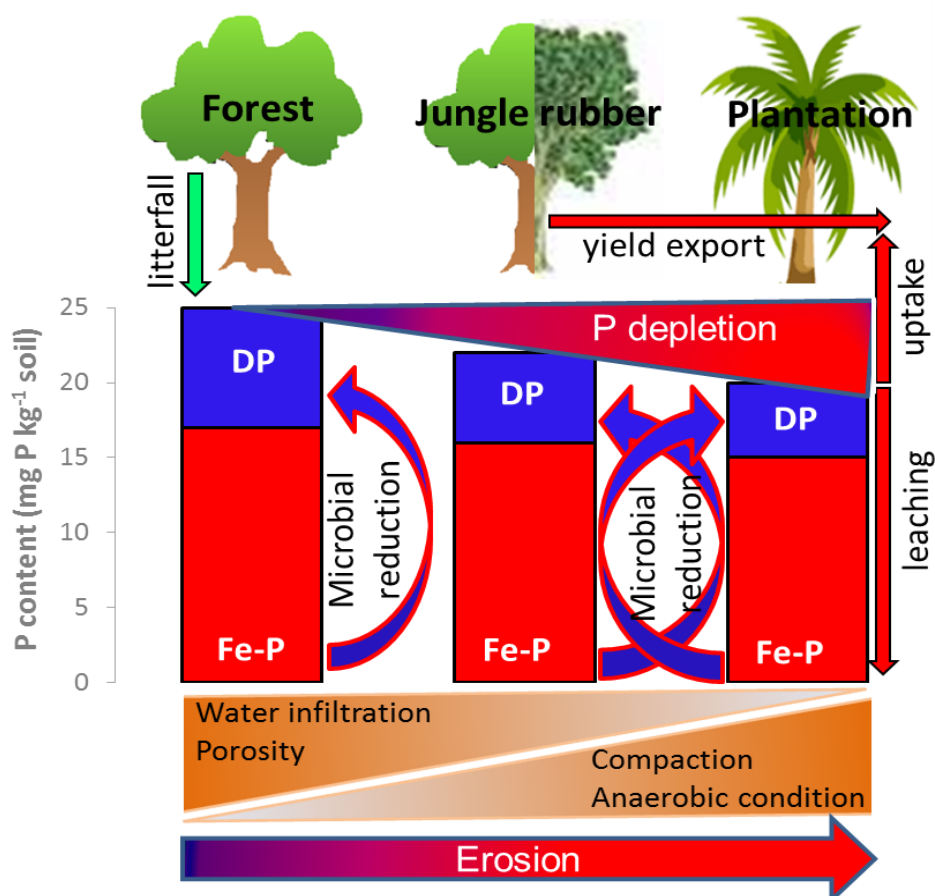
Long-term incubation showed that iron dissolution and P mobilization were reversible processes. In the second phase, after three weeks, P availability in soils decreased, although Fe-P (Fig. 4) and available P (Fig. 2) did not reach initial levels. The decrease of  $D\text{Fe}^{2+}$  (Fig. 1) indicates that microbial reduction of  $\text{Fe}^{3+}$  eventually stopped or was strongly reduced and that the produced  $\text{Fe}^{2+}$  underwent secondary chemical reactions, which lead to the precipitation of  $\text{Fe}^{2+}$ . Our results confirmed the findings of Wood et al. (2015), in which soil solution P increase after flooding is only temporary and restricted to the early reducing conditions, after which it decreased again. We hypothesized that some of the P released via  $\text{Fe}^{3+}$  reduction would be re-adsorbed to freshly precipitated  $\text{Fe}^{2+}$  oxides because of their larger adsorptive surface area per unit soil volume (Chacon et al., 2006; Wright et al., 2001). This process, however, was not significant because DP did not decrease following  $\text{Fe}^{2+}$  precipitation. We were unable to identify the exact cause of changes in  $\text{Fe}^{3+}$  reduction and P availability, but it was not related to changes in soil pH. One potential explanation is an exhaustion of easily available C sources for microbial reduction. Indeed, DOC concentrations (Fig. 5) decreased in a pattern similar to  $D\text{Fe}^{2+}$  concentrations (Fig. 1). In conclusion, even if the increase of available P fractions in soils after flooding is fast but reversible, the P released during initial flooding is not, as long as flooding lasts. Finally, the second phase might only rarely occur in natural conditions because 1) water movements would prevent the establishment of the physico-chemical conditions favoring precipitation and 2) C input by rhizodeposition would prevent C limitation of the microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  minerals.

### *Ecological relevance*

In most tropical soils, P is widely believed to limit plant growth (Cleveland et al., 2006; Townsend et al., 2002; Vitousek, 1984). This is both because of the relatively low total soil P concentrations and because the high Fe and Al oxide

content causes a strong fixation of plant available inorganic P into forms unavailable for biota (Bucher et al., 2001; Holford, 1997; Maranguit et al., 2017). Microbially-mediated  $\text{Fe}^{3+}$  reduction and the associated P release during anaerobic conditions play a fundamental role in the nutrition of plants and microorganisms by releasing inaccessible P during dry conditions. This mechanism is relevant not only in riparian areas or paddy soils, where flooding occurs, but also in well-drained soil partly waterlogged by regular heavy rainfalls during the wet season.

Surprisingly, intensive rubber and oil palm plantations were already strongly depleted in their most refractory P pool a decade after forest conversion and despite P fertilization (Maranguit et al. 2017). This unexpected fast effect of land-use changes on the presumably least reactive P fractions could be driven by a synergy between increased P mobilization by microbially-mediated  $\text{Fe}^{3+}$  reduction and a higher P output. On one hand, rubber and especially oil palm plantations suffer from soil compaction, resulting in higher bulk density and lower water infiltration and leading to strong erosion (Guillaume et al., 2016; Merten et al., 2016). Consequently, these intensive plantations are more subject to waterlogging and lengthier anaerobic conditions than are more porous soils (e.g., forest and jungle rubber); this makes the cycle turnover of P availability faster (Fig. 7). On the other hand, mobilized P can be exported from the site through runoff/erosion in the plantation. Indeed, strong soil erosion was estimated under rubber and oil palm plantations (Guillaume et al., 2015). Another very important output of mobilized P is through harvested biomass or leaching in groundwater if the P sink capacity of  $\text{Fe}^{3+}$  oxide in the subsoil is reduced due to  $\text{Fe}^{3+}$  reduction. Oil palms have a dense rooting system down to 30 cm depth, but fewer roots than forest in deep soils (Kotowska et al., 2015; Nelson et al., 2006). Hence, oil palms might be less efficient than forest in pumping P mobilized during waterlogged conditions in deep soil, leading to P losses (Fig. 7).



**Figure 7** Conceptual figure showing that reducing conditions mobilize bound P through microbially-mediated Fe reduction and P dissolution making P available for plant uptake. In plantations, anaerobic conditions are more frequent and longer: this increases P availability, accelerates the P cycling, and increased the P uptake by vegetation. Three important P losses are responsible for P depletion: (1) biomass export with yield; (2) soil runoff/erosion and (3) P leaching if the vegetation cannot pump it because of fewer roots in deep soils or less active roots because of anaerobic conditions.

### 2.3.6 Conclusions

The increase of available P and DP during flooding is due to three main mechanisms that were strongly influenced by land-use types: (1) P release via the microbially-mediated reductive dissolution of  $\text{Fe}^{3+}$  oxides; (2) P release during SOM mineralization and (3) solubility of Fe phosphate due to increasing pH.

$\text{Fe}^{3+}$  reduction and P release were pronounced in topsoil compared to subsoil and always higher in forest and jungle rubber than in monoculture plantations throughout the flooding period. The maximum increase of P concentration was in the early stages of flooding, simultaneously with peak  $\text{DFe}^{2+}$  and DOC

in the soil solution. The increase of available P and DP in the topsoil of forest and jungle rubber was about 50-70% of the initial concentration before flooding. Consequently, flooding contributed to P mobilization and thus to its potential uptake by plant roots.  $\text{Fe}^{3+}$  reduction by microorganisms was the key process behind the P release. Nonetheless, the extent of the microbially-mediated reductive dissolution of ferric oxides was apparently determined by the SOM content because it is a source of C and energy that enables microorganisms to efficiently reduce  $\text{Fe}^{3+}$ . Hence, soils from forest and jungle rubber, which contains high SOM, resulted in a faster and higher P release than the plantation soils. Apart from being an energy source for microorganisms, the mineralization of SOM also contributes to the increase of available P. The P release was higher only in the early stage of flooding across all land-uses and depths. The increasing pH in topsoil and subsoil after flooding of all land-use types also influence the P release. After three weeks of flooding, both P availability in soils and  $\text{DFe}^{2+}$  decreased. This indicates that microbial  $\text{Fe}^{3+}$  reduction stopped or was strongly reduced and that the  $\text{Fe}^{2+}$  produced underwent secondary chemical reactions, leading to the precipitation of  $\text{Fe}^{2+}$ . Nonetheless, the short-term P release via  $\text{Fe}^{3+}$  reduction provided an important source of available P in flooded ecosystems and in well-drained, highly weathered soil partly waterlogged after seasonal flooding. Anaerobic conditions occur more often in compacted plantation soils compared to forest, and so, the increase P availability under plantations. This accelerates the P cycling: more P is pumped by the vegetation. Three important P losses are responsible for P depletion: (1) soil runoff/erosion; (2) biomass export from yield and (3) P leaching.

### 2.3.7 Acknowledgements

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## 2 Publications and Manuscripts: Study 3

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**Table A.1.** Influence of land-use on the change in DP, DFe<sup>2+</sup> and DOC of topsoil between two time points as indicated by the p-value of significance evaluated using a Repeated measures ANOVA model.

Time	Dissolved P			Dissolved Fe <sup>2+</sup>			Dissolved Org C		
	LU <sup>a</sup>	Δtime <sup>b</sup>	LU*Δtime <sup>c</sup>	LU	Δtime	LU*Δtime	LU	Δtime	LU*Δtime
0	-	-	-	-	-	-	-	-	-
7	0.108 <sup>ns</sup>	0.000 <sup>***</sup>	0.212 <sup>ns</sup>	0.498 <sup>ns</sup>	0.000 <sup>***</sup>	0.488 <sup>ns</sup>	-	-	-
14	0.041 <sup>*</sup>	0.978 <sup>ns</sup>	0.001 <sup>***</sup>	0.295 <sup>ns</sup>	0.000 <sup>***</sup>	0.352 <sup>ns</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.059 <sup>ns</sup>
21	0.009 <sup>**</sup>	0.024 <sup>*</sup>	0.085 <sup>ns</sup>	0.000 <sup>***</sup>	0.032 <sup>*</sup>	0.604 <sup>ns</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>	0.000 <sup>***</sup>
30	0.012 <sup>*</sup>	0.009 <sup>**</sup>	0.015 <sup>*</sup>	0.037 <sup>*</sup>	0.053 <sup>ns</sup>	0.952 <sup>ns</sup>	0.000 <sup>***</sup>	0.006 <sup>**</sup>	0.042 <sup>*</sup>
45	0.009 <sup>**</sup>	0.531 <sup>ns</sup>	0.447 <sup>ns</sup>	0.254 <sup>ns</sup>	0.017 <sup>*</sup>	0.993 <sup>ns</sup>	0.000 <sup>***</sup>	0.126 <sup>ns</sup>	0.791 <sup>ns</sup>
60	0.018 <sup>*</sup>	0.596 <sup>ns</sup>	0.651 <sup>ns</sup>	0.559 <sup>ns</sup>	0.002 <sup>**</sup>	0.240 <sup>ns</sup>	0.000 <sup>***</sup>	0.130 <sup>ns</sup>	0.089 <sup>ns</sup>

<sup>a</sup> p-value within land-use effect

<sup>b</sup> p-value of the changes between two time points (e.g., between day 0 and 7; corresponding p-value indicated on day 7)

<sup>c</sup> p-value of the influence of land-use on the change of parameters between two time points

### 3 Additional studies

#### 3.1 Study 4: Sensitivity and resistance of soil fertility indicators to land-use changes: New concept and examples from conversion of Indonesian rainforest to plantations

Thomas Guillaume<sup>a,b,c,\*</sup>, Deejay Marangit<sup>a</sup>, Kukul Murtilaksono<sup>d</sup>, Yakov Kuzyakova<sup>e</sup>

Status: Published online in *Ecological Indicators*

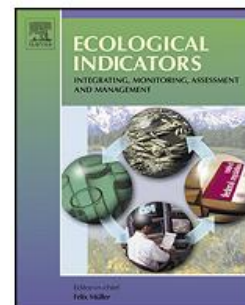
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##### 3.1.1 Abstract

Tropical forest conversion to agricultural land leads to a strong decrease of soil organic carbon (SOC) stocks. While the decrease of the soil C sequestration function is easy to measure, the impacts of SOC losses on soil fertility remain unclear. Especially the assessment of the sensitivity of other fertility indicators as related to ecosystem services suffers from a lack of clear methodology. We developed a new approach to assess the sensitivity of soil fertility indicators and tested it on biological and chemical soil properties affected by rainforest conversion to plantations. The approach is based on (non-)linear regressions between SOC losses and fertility indicators normalized to their level in a natural ecosystem. Biotic indicators (basal respiration, microbial biomass, acid phosphatase), labile SOC pools (dissolved organic carbon and light fraction) and nutrients (total N and available P) were measured in Ah horizons from rainforests, jungle rubber, rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*) plantations located on Sumatra. The negative impact of land-use changes on all measured

indicators increased in the following sequence: forest < jungle rubber < rubber < oil palm. The basal respiration, microbial biomass and nutrients were resistant to SOC losses, whereas the light fraction was lost stronger than SOC. Microbial C use efficiency was independent on land use. The resistance of C availability for microorganisms to SOC losses suggests that a decrease of SOC quality was partly compensated by litter input and a relative enrichment by nutrients. However, the relationship between the basal respiration and SOC was non-linear; i.e. negative impact on microbial activity strongly increased with SOC losses. Therefore, a small decrease of C content under oil palm compared to rubber plantations yielded a strong drop in microbial activity. Consequently, management practices mitigating SOC losses in oil palm plantations would strongly increase soil fertility and ecosystem stability. We conclude that the new approach enables quantitatively assessing the sensitivity and resistance of diverse soil functions to land-use changes and can thus be used to assess resilience of agroecosystems with various use intensities.

**Keywords:** SOC availability, Soil degradation, Land use, Microbial activity, Labile pool, Ecosystem resistance

### 3.2 Study 5: Effects of P and C inputs on microbial activities in P limiting bulk and rhizosphere soil

Nataliya Bilyera<sup>a,\*</sup>, Evgenia Blagodatskaya<sup>b</sup>, **Deejay Maranguit**<sup>b,c</sup>, Yakov Kuzyakov<sup>b</sup>

Status: In preparation

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#### 3.2.1 Abstract

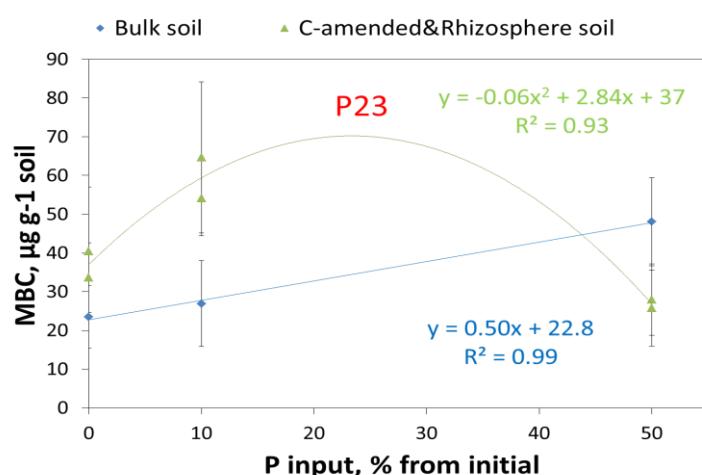
Phosphorus (P) is the second important nutrient for plants and limiting element in many ecosystems. P is a nonrenewable resource, and based on its current rate of use, it has been estimated that the worlds known reserves of P rocks may be depleted within the current century. Soils with high-sorption P capacity require higher P additions, but, do not provide plants with sufficient available P. Therefore, it is necessary to reduce P application rates, but facilitate soil microbiological activity to maintain good P availability for plants.

We aimed to study soil adenosine triphosphate (ATP), microbial biomass (MBC) and phosphatase activity as microbial response to contrasting P input in a low P Cambisol in a 5 days incubation experiment. The treatments were i) bulk soil (no C), ii) rhizosphere soil (10 µg C g<sup>-1</sup> soil day<sup>-1</sup> – root exudates imitation) and iii) glucose addition to soil (50 µg C g<sup>-1</sup> soil – for microbial activation). Three rates of P as KH<sub>2</sub>PO<sub>4</sub> were applied at each C treatments: i) no P (P<sub>0</sub>) – for P severe limitation; ii) 10% P from initial extractable soil P (P<sub>10</sub>) – low P input; and iii) 50% P from initial extractable soil P (P<sub>50</sub>) – high P input. We tested the following hypotheses: 1) the better response of MBC and ATP to P is expected to be in the rhizosphere soil, as continuous C input resulted in gradual microbial activation; 2) phosphatase activity will decrease with increasing P rates in all soils. Microbial biomass grew linear ( $R^2 = 0.99$ ) and simultaneously with incremental P addition in bulk soil. In rhizosphere and C-amended soils, on contrary, the MBC response to P level was represented by quadratic model ( $y = -0.06x^2 + 2.84x + 37.03$ ;  $R^2 = 0.93$ ). This model shows the

highest MBC value at P23, which indicates optimal and the most effective application rate for this soil type. The correlation between soil ATP content and P rates ascended in the order bulk soil ( $R^2 = 0.34$ ) > C-amended soil ( $R^2 = 0.51$ ) > rhizosphere soil ( $R^2 = 0.97$ ). That proves our hypothesis that continuous C input (similar to root exudations) stimulates gradual microorganism activation. The soil ATP content per gram of microbial biomass C increased linearly ( $y = 5.09x + 21.4$ ;  $R^2 = 0.99$ ) with increasing P rates in rhizosphere, whereas in bulk and C-amendment soils the effect of P was less pronounced. Phosphatase activity declined (57 and 64%) exponentially with increasing P rates for rhizosphere ( $R^2 = 0.84$ ) and C-amended ( $R^2 = 0.98$ ) soils, that complies with our hypothesis. In bulk soil, on contrary, phosphatase activity increased (35%) at P<sub>10</sub> and remained constant at P<sub>50</sub>. P<sub>0</sub> was resulted in 5-folds higher phosphatase activity in rhizosphere and C-amended soils compared to bulk soil. This proves the significance of root exudates in facilitation of microbial phosphatase production. Our results show that P (re)cycling can be accelerated in P-deficient soils by C addition and so, excessive P fertilization can be avoided to maintain ecosystem sustainability.

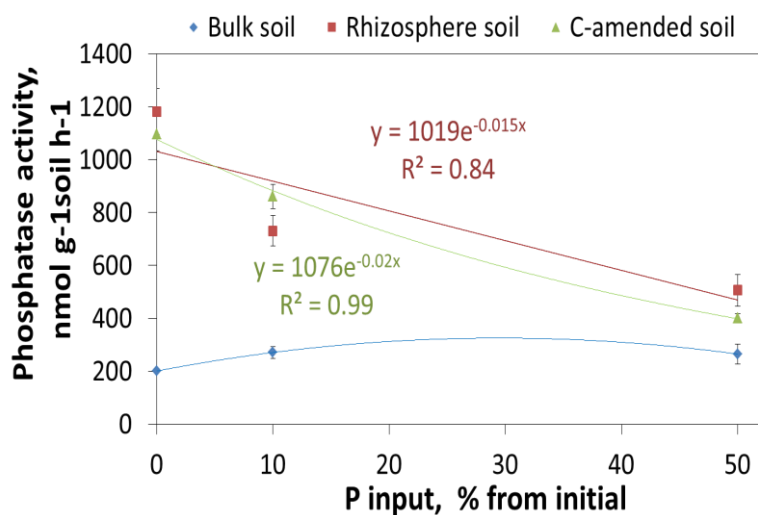
**Keywords:** phosphorus, soil ATP, phosphatase, microbial biomass, Cambisol.

### 3.2.2 Highlights



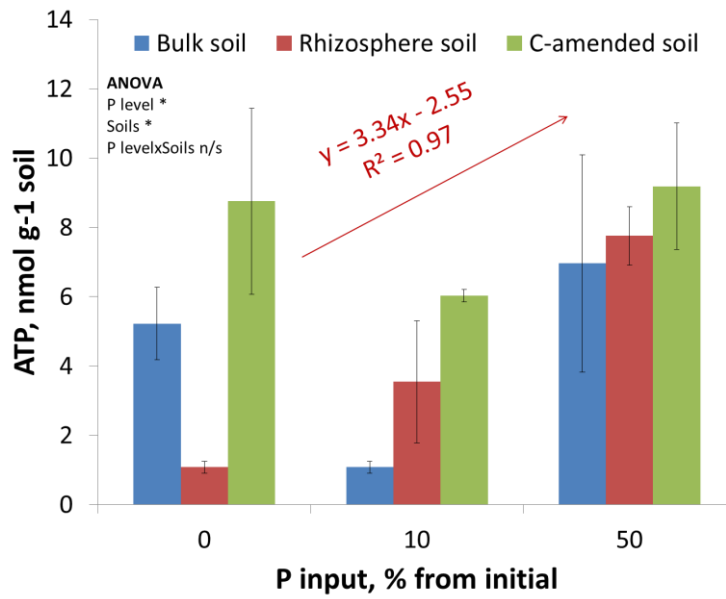
**Figure 1** Microbial biomass carbon ( $\mu\text{g g}^{-1}$  soil) after five days incubation of bulk, rhizosphere and C-amended soil with different rate of P.

Microbial biomass grew linear ( $R^2 = 0.99$ ) and simultaneously with incremental P addition in bulk soil. In rhizosphere and C-amended soils, on contrary, the MBC response to P level was represented by quadratic model( $y = -0.06x^2 + 2.84x + 37.03$ ;  $R^2 = 0.93$ ).

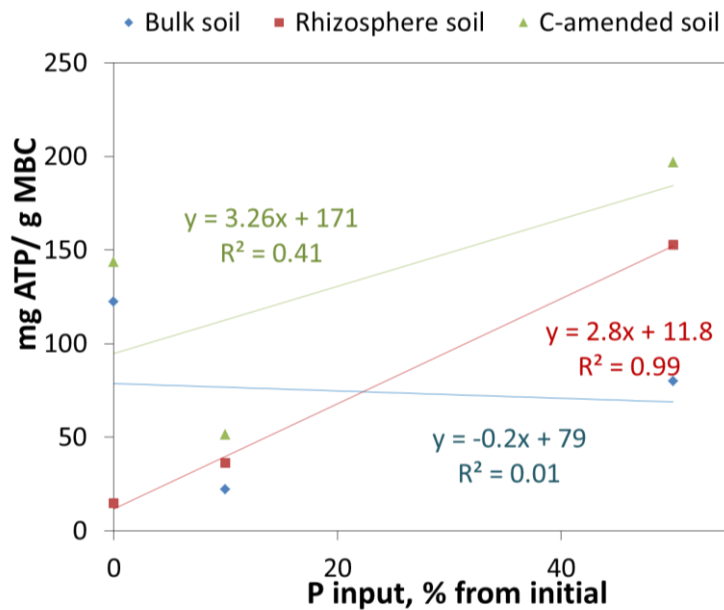


**Figure 2** Phosphatase activity (nmol g<sup>-1</sup> soil h<sup>-1</sup>) after five days incubation of bulk, rhizosphere and C-amended soil with different rate of P fertilizer.

Phosphatase activity declined (57 and 64%) exponentially with increasing P rates for rhizosphere ( $R^2 = 0.84$ ) and C-amended ( $R^2 = 0.98$ ) soils. In bulk soil, on contrary, phosphatase activity increased (35%) at P<sub>10</sub> and remained constant at P<sub>50</sub>. P<sub>0</sub> was resulted in 5-folds higher phosphatase activity in rhizosphere and C-amended soils compared to bulk soil.



**Figure 3** Soil ATP content (nmol g<sup>-1</sup> soil) after five days incubation of bulk, rhizosphere and C-amended soil with different rate of P fertilizer.



**Figure 4** Soil ATP content per gram of microbial biomass C (mg ATP g<sup>-1</sup> MBC) after five days incubation of bulk, rhizosphere and C-amended soil with different rate of P fertilizer.

ATP increased linearly ( $y = 5.09x + 21.4$ ;  $R^2 = 0.99$ ) with increasing P rates in rhizosphere, whereas in bulk and C-amendment soils the effect of P was less pronounced.

### **3.3 Study 6: Influence of earthworm on microbial biomass P and phosphatase activity**

Status: In preparation

#### **3.3.1 Background and objective of the study**

Earthworms are playing a crucial role in nutrient cycling. They influence the supply of nutrients through their tissues but largely through their burrowing activities; they produce aggregates and biopores in the soil and/or on the soil surface, thus affecting its physical properties, nutrient cycling, and plant growth. Earthworms, bury above ground litter, produces cast and mucus that enhances the activity of beneficial soil microorganisms colonizing in the biopores.

In soil, phytate is the most abundant and recalcitrant form of organic P (Daniel et al., 2013). This is hydrolyzed by phosphatase enzymes to form available P for microbial and plant growth. Acid phosphatase in soil is produced by both plants and microorganisms (fungi, bacteria) (Turner et al., 2002; Lee et al., 2008; Nannipieri et al., 2011). Phosphatase activity is increased by the colonization of biopores by P-solubilizing bacteria (Wan et al., 2004). Furthermore, since the digestive gut of earthworms secretes phosphatase, an increase of phosphatase activity could be predicted after soil has passed through the gut (Vinotha et al., 2000). Enzyme activities in burrow walls would be expected to reflect interactions between earthworms and microorganisms and clarify the role of earthworms in soil nutrient cycles.

The objective of this study was to investigate microbial activity controlling P-availability such as P uptake of microorganisms (microbial biomass P content [MBP]) and phosphatase activity in biopores.

#### **3.3.2 Methodology**

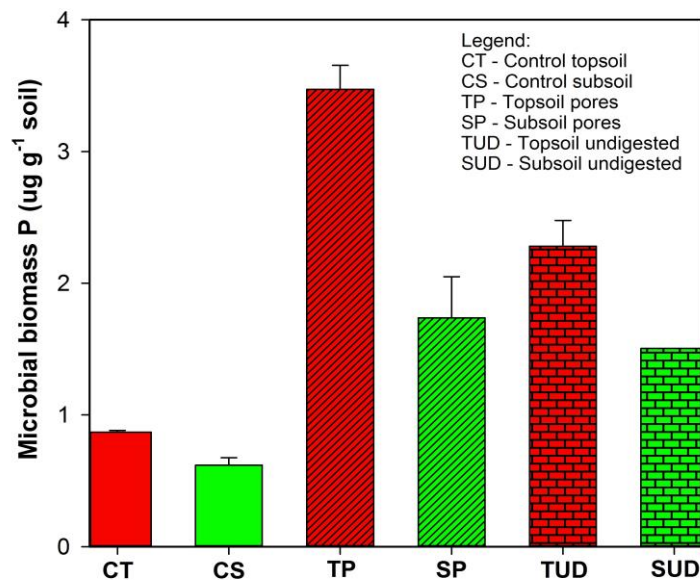
Briefly, topsoil and subsoil from agriculture field were packed in each of the four rhizoboxes. Leaf litters were put above the topsoil. Earthworms were kept above the leaf litters in each rhizobox. Three control rhizoboxes without earthworms were also included. After 3 months incubation, microbial biomass



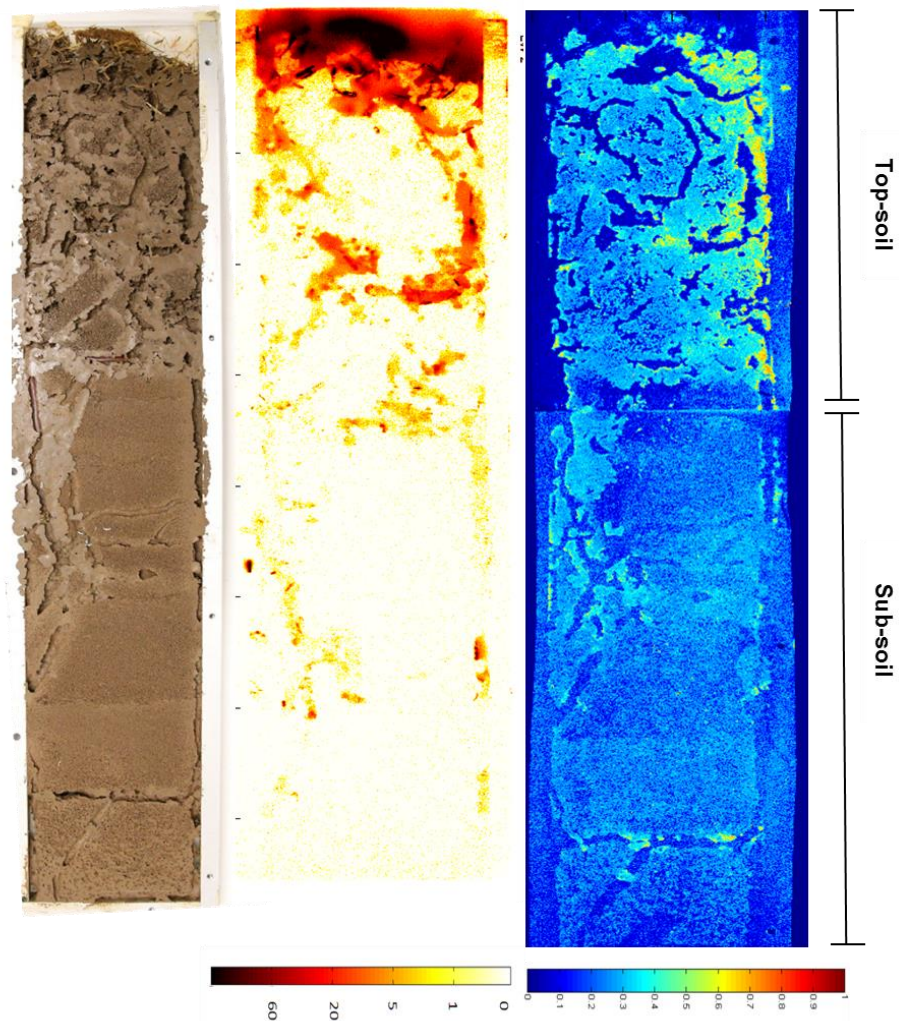
P in control soil, in topsoil and subsoil pores and in undigested topsoil and subsoil were determined following chloroform-fumigation method (Hedley et al., 1982). Phosphatase activity was determined in-situ by Zymography.

### 3.3.3 Highlights

The results of the study suggest that earthworms do influence P availability. The MBP content was highest in soils from pores (both topsoil and subsoil) compared to control soil without earthworms and to undigested soils (Fig. 1). This increased in MBP content in pores has a relevant contribution to the available P content after microbial biomass turnover.

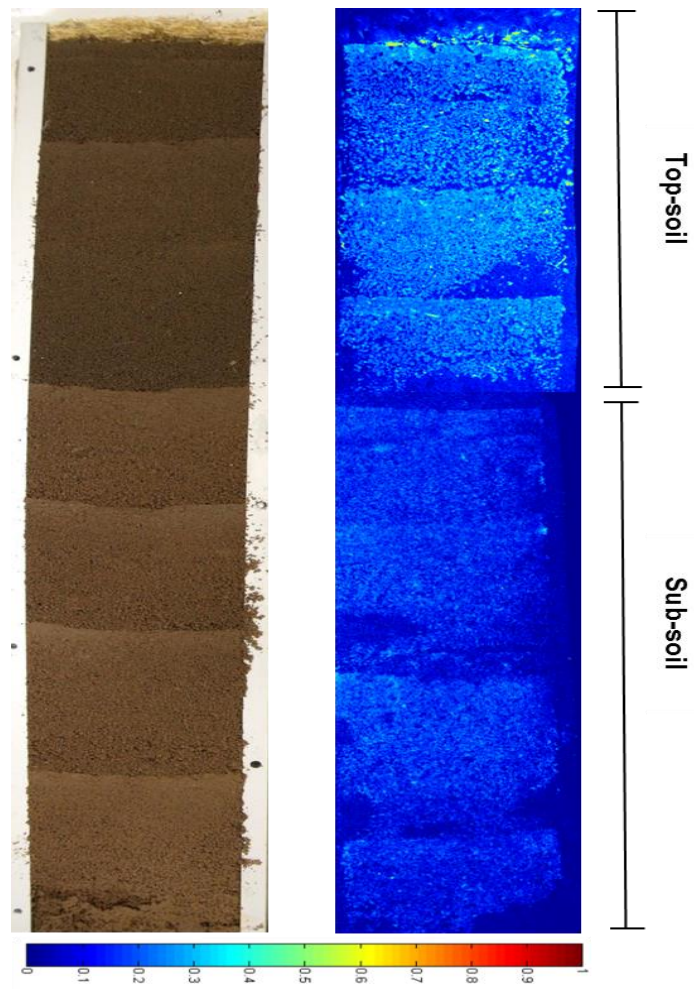


**Figure 1** Microbial biomass P ( $\mu\text{g g}^{-1}$  soil) in control, pores and undigested topsoil and subsoil. Values represent means  $\pm$  SE ( $n=4$ ).

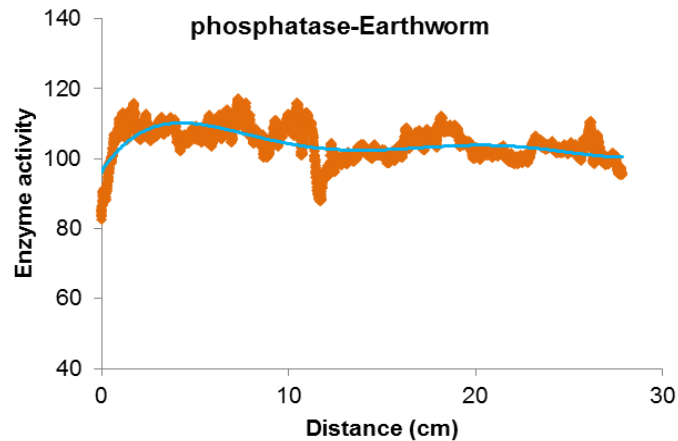


**Figure 2** a) examples of earthworms in rhizobox b) radioisotope image ( $^{14}\text{C}$ ) and c) zymographs showing spatial distribution of enzyme activities. Side colormaps are proportional to the  $^{14}\text{C}$  activity (KBq) and MUF calibration line.

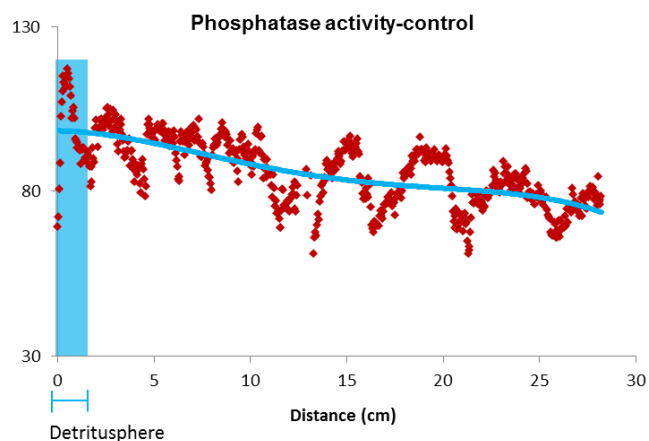
The 2D-images revealed that a) earthworms distribute litter and C in top- and sub-soil, b) enzyme activity was mainly associated with the biopore and detritusphere. This high activity is primarily attributed to the inputs of easily degradable organic compounds from the earthworm casts, litters and resulting stimulation of microorganisms, and the direct release of enzymes by earthworms.



**Figure 3** a) examples of control rhizobox without earthworms and b) corresponding zymogram. High phosphatase activity attributed to the detritusphere is visible in the top of control box.



**Figure 4** Phosphatase activity is accelerated in top- and sub-soil under effect of earthworms.



**Figure 5** Phosphatase activity is accelerated in vicinity of detritusphere created by plant residue. Thereafter phosphatase activity slightly declined from top- to sub-soil.

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### **3.4 Study 7. Effect of land-use change on P availability in sub-tropical soil in Nepal**

Status: In Preparation

#### **3.4.1 Background and objective of the study**

The increasing population in Nepal, for example in Chitwan districts lying in Terai region with current population growth rate of 1.75% the highest in Nepal, is continuously giving pressure to forest areas (Maharjan et al., 2017, FRA/DFRS, 2014 ). Massive deforestation and conversion to agricultural land-use occurred in order to meet food demand in the region. However, consequences to soil P were never given attention. Therefore, the objective of the study was to investigate the effects of land-use conversion on P and its various fractions of different availability.

#### **3.4.2 Methodology**

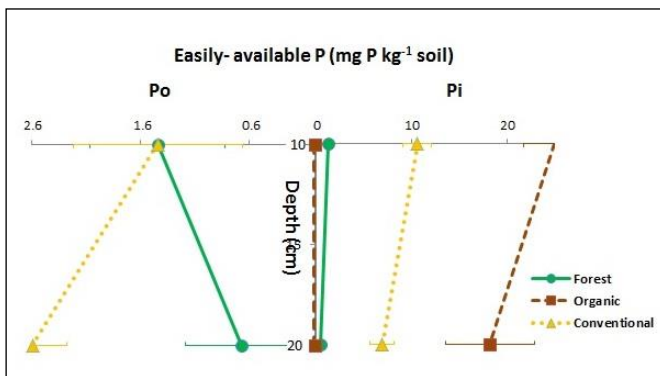
Soil samples were collected in Chitwan district (27° 35'N 84° 30'E) of Nepal in three land-use types: forest, organic and conventional farming. Samples were collected in topsoil (0–10 cm) and subsoil (10–20 cm). Fresh samples were brought to the laboratory of the Department of Soil Science in Temperate Ecosystem in Göttingen University, Germany for P fractionation and other further analyses. Phosphorus fractionation was done following Hedley et al. (1982) P sequential fractionation method. Phosphate in every fraction was quantified following malachite green colorimetric method (D'Angelo et al., 2001)

#### **3.4.3 Highlights**

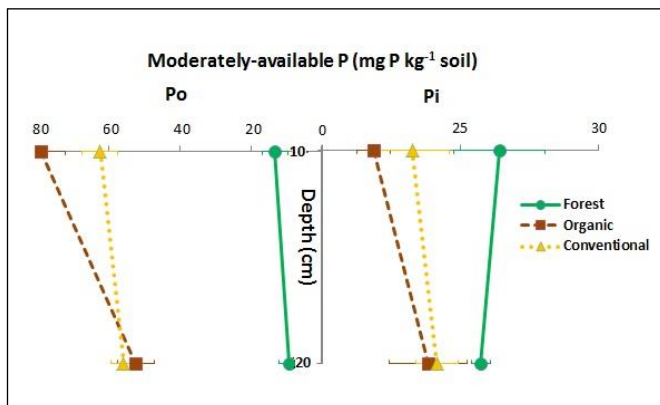
Forest conversion to organic and conventional farming in Chitwan district of Nepal affects the forms, distribution and availability of P in the soil. The easily-available  $P_i$  content in organic and conventional farming was higher compared to forest soil. This is due to the application of organic residues and inorganic P fertilizer in organic and conventional farming, respectively. Unexpectedly, the easily-available  $P_o$  content in organic farming was found to be very low (Fig. 1). Probably, there was a fast decomposition and mineralization of easily-

available Po in the organic farming system, contributing to the easily-available Pi content. Aside from fast mineralization of easily-available Po, most of Po in organic farming is in moderately-available (topsoil  $\approx 80$  mg P; subsoil  $\approx 60$  mg P  $\text{kg}^{-1}$  soil; Fig. 2) and non-available form (topsoil  $\approx 250$  mg P; subsoil  $\approx 150$  mg P  $\text{kg}^{-1}$  soil; Fig. 3). Consequently, in the long-run, moderately-available Po form sustains long-term P fertility after mineralization buffering easily-available Pi.

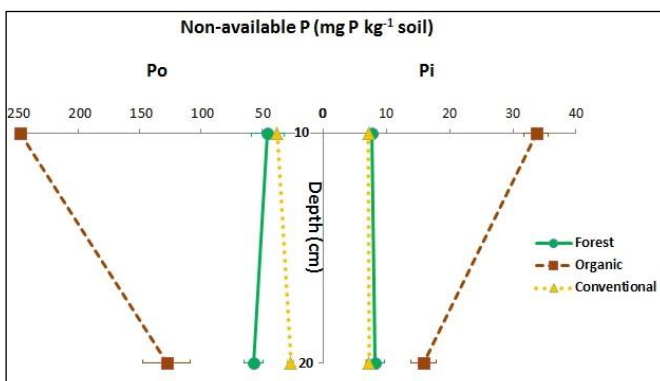
On the other hand, forest soil has the lowest content of easily-available P, obviously because it was never fertilized. Most of the P was bound with Fe oxide (moderately-available form). Nonetheless, it is very important in sustaining long-term P fertility after desorption.



**Figure 1** Easily-available Po (left) and Pi (right) (mg P kg<sup>-1</sup> soil) in soil under different land-use types extracted by 0.5 M NaHCO<sub>3</sub>.



**Figure 2** Moderately-available Po (left) and Pi (right) (mg P kg<sup>-1</sup> soil) in soil under different land-use types extracted by 0.1 M NaOH.



**Figure 3** Non-available Po (left) and Pi (right) (mg P kg<sup>-1</sup> soil) in soil under different land-uses types extracted by 1 M HCl.

### 3.4.4 References

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## 4 Appendix

### 4.1 Contributions to the studies

This PhD thesis comprises seven studies which were successfully done in cooperation with several coauthors. The contributions of these coauthors are as follows:

#### **Study 1. Biotic and abiotic processes of phosphorus cycling in acidic P-limited soil: $^{33}\text{P}$ tracer assessment**

Status: Under review in *Biology and Fertility of Soil* (since March 2017)

Deejay Maranguit:	60% (experimental design, accomplishment of experiment, laboratory analyses, data preparation and interpretation, manuscript preparation)
Nataliya Bilyera:	30% (experimental design, discussion of manuscript structure, comments to improve the manuscript)
Yakov Kuzyakov:	10% (discussion of manuscript structure; comments to improve the manuscript)

#### **Study 2. Land-use change affects phosphorus fractions in highly weathered tropical soils**

Status: Published online in *Catena*

Deejay Maranguit:	70% (data collection, analysis and interpretation; manuscript preparation)
Thomas Guillaume:	20% (soil collection, discussion of manuscript structure; comments to improve the manuscript)
Yakov Kuzyakov:	10% (discussion of manuscript structure; comments to improve the manuscript)

#### **Study 3. Effects of flooding on phosphorus and iron mobilization in highly weathered soils under different land-use types: Short-term effects and mechanisms**

Status: Under review in *Catena* (since March 2017)

Deejay Maranguit:	70% (data collection, analysis and interpretation; manuscript preparation)
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Thomas Guillaume:	20% (soil collection, discussion of manuscript structure; comments to improve the manuscript)
Yakov Kuzyakov:	10% (discussion of manuscript structure; comments to improve the manuscript)

**Study 4. New approach for sensitivity of soil fertility indicators to land-use changes: Theory and examples from conversion of Indonesian rainforest to plantations**

Status: Published online in *Ecological Indicators*

Thomas Guillaume:	50% (soil sampling, experimental design, accomplishment of experiment, data preparation and interpretation, manuscript preparation)
Deejay Maranguit:	20% (laboratory analyses, comments to improve the manuscript)
Kukuh Murti Laksono:	10% (comments to improve the manuscript)
Yakov Kuzyakov:	20% (experimental design, data interpretation, discussion of manuscript structure, comments to improve the manuscript)

**Study 5. Effects of P and C inputs on microbial activities in P limiting bulk and rhizosphere soil**

Status: In preparation

Nataliya Bilyera:	50% (experimental design, accomplishment of experiment, data preparation and interpretation, manuscript preparation)
Evgenia Blagodatskaya:	20% (experimental design, interpretation of data, comments to improve the manuscript)
Deejay Maranguit:	20% (laboratory analyses, data preparation and interpretation, comments to improve the manuscript)
Yakov Kuzaykov:	10% (interpretation of data, comments to improve the manuscript)

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**Study 6. Influence of earthworm on microbial biomass P and phosphatase activity**

Status: In preparation

Duyen Hoang: 40% (experimental design, accomplishment of the experiment, data preparation and interpretation, manuscript preparation)

Bahar Rasavi: 30% (experimental design, accomplishment of the experiment, data preparation and interpretation, comments to improve the manuscript)

Deejay Maranguit: 20% (laboratory analyses, data preparation and interpretation)

Yakov Kuzyakov: 10% (discussion on experimental design)

**Study 7. Effect of land-use change on P availability in sub-tropical soil in Nepal**

Status: In preparation

Menuka Maharjan: 50% (experimental design, accomplishment of the experiment, data preparation and interpretation, manuscript preparation)

Deejay Maranguit: 45% (laboratory analyses, data interpretation, discussion of manuscript structure)

Yakov Kuzyakov: 5% (discussion on experimental design)

## 4.2 Declarations

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form.

Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

Göttingen,

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(Signature)

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorized aid.

Göttingen,

.....

(Signature)

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### 4.3 Curriculum vitae

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**Name:** Deejay S. Maranguit                      **E-mail:** maranguitdeejay@gmail.com  
**Date of Birth:** November 29, 1988              **Tel:** +49 162 829 6906  
**Place of Birth:** Leyte                              **Nationality:** Philippines

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#### Education Career

11.2014 – 2017                      PhD Candidate; University of Gottingen, Germany  
 Biodiversity and ecology

06.2010 – 04.2012                      Master of Soil Science; Minor in Tropical ecology  
 Visayas State University, Philippines  
*Thesis: Characteristics and origin of soils along the central  
 mountain range in Leyte*

06.2005 – 04.2009                      Bachelor of Science in Agriculture; Major in Soil  
 Science; Visayas State University, Philippines  
*Thesis: Growth performance and nitrogen uptake of pechay  
 (Brassicus napus L.) grown in acid soil amended with  
 NPK inorganic fertilizer combined with varying levels of  
 vermicast*

06.2001 – 03.2005                      High School; Visayas State University, Laboratory  
 High School, Philippines

#### Professional Experiences

07.2016 – Present                      University Instructor III; Visayas State University

06.2012 – 06.2016                      University Instructor I; Visayas State University

01.2010 – 06.2010                      Science Research Assistant; Visayas Consortium for  
 Agriculture and Resources Program (ViCARP)

11.2009 – 12.2009                      Soil Analyst; Australian Centre for International  
 Agricultural Research (ACIAR-FORESTRY)

05.2009 – 10.2009                      Supervisor; ComVal Tropical Fruit Inc.

## Honours and Awards

2017	2 <sup>nd</sup> Best paper award senior category (PSSST 2017)
2016	1 <sup>st</sup> Best paper award senior category (PSSST 2016)
2014 – 2017	Recipient, Experts4Asia Erasmus mundus PhD scholarship
2010 – 2012	Recipient, Accelerated Science and Technology Human Resource Development Program-National Science Consortium Graduate Scholarship of DOST
2010	Recipient, National Chung Hsing University Scholarship for graduate study at NCHU, Taiwan (withdrew in favour of another scholarship award)
2010 – 2011	Dean's Honor for outstanding academic performance during the MS program
2007 – 2009	Recipient, Dr. Fernando Bernardo Scholarship (3 <sup>rd</sup> to 4 <sup>th</sup> year in Bachelor)
2005 – 2006	Recipient, VSU university scholarship (1 <sup>st</sup> to 2 <sup>nd</sup> year in Bachelor)

## Expertise

Soil fertility, Nutrient Cycling, Land-use, Pedology, Soil ecology, Phosphorus cycling, Soil phosphorus quantification

## Publications

- Maranguit D.M.\*, Guillaume T., Kuzyakov Y., 2017. Land-use change affects phosphorus fractions in highly weathered tropical soils. *Catena* 149, 385-393. doi: 10.1016/j.catena.2016.10.010.
- Guillaume T.\*, Maranguit D.S., Mortilaksono K., Kuzyakov, Y., 2016. Sensitivity and resistance of soil fertility indicators to land-use changes: New concept and examples from conversion of Indonesian rainforest to plantations. *Ecological Indicators* 67, 49-57. doi.org/10.1016/j.ecolind.2016.02.039.
- Asio V.B.\* , Lina S.B., Maranguit D.S., Bolledo A.B., Doguiles R., Quinones C.M.O., Sabijon J., Demain K.L., 2014. Characteristics of soils in marginal uplands of Inopacan Leyte. *Annals of Tropical Research* 36, 1-15
- Lina S.B.\* , Maranguit D.S., Asio V.B., Sabijon J.R., Demain K.L., Bolledo A.B., 2014. Growth Performance of Corn as Influenced by Combined Application of Organic and Inorganic Fertilizers in Highly Degraded Soil. *Annals of Tropical Research* 36, 16-29

- Maranguit D.S.\*, Asio V.B., 2013. Morpho-physical and Chemical Characteristics of soils along the central mountain range in Leyte. *Annals of Tropical Research* 35,35-60
- Maranguit D.S.\*, Guillaume T., Kuzyakov Y., 2017. Effects of flooding on phosphorus and iron mobilization in highly weathered soils under different land-use types: Short-term effects and mechanisms. *Catena* (accepted)
- Maranguit D.S.\*, Bilyera N., Kuzyakov Y., 2017. Biotic and abiotic processes of phosphorus cycling in acidic P-limited soil: <sup>33</sup>P tracer assessment. *Biol Fertil Soils* (submitted)

### Conference Papers

- European Geosciences Union (EGU) 2017: “*Effects of flooding on phosphorus and iron mobilization in highly weathered soils: Short-term effects and mechanisms*”. Maranguit D.S., Guillaume T., Kuzyakov Y. **poster presentation**
- European Geosciences Union (EGU) 2016: “*Land-use intensification impact on P fractions in highly weathered tropical soils*”. Maranguit D.S., Guillaume T., Kuzyakov Y. **poster presentation**
- Philippine Society of Soil Science and Technology (PSSST) 2017: “*<sup>33</sup>P tracer assessment on the fate of P fertilizer*”. Maranguit D.S., Bilyera N., Kuzyakov Y. **oral presentation**
- Philippine Society of Soil Science and Technology (PSSST) 2017: “*Does soil flooding facilitate phosphorus and iron mobilization in highly weathered soils?*”. Maranguit D.S., Guillaume T., Kuzyakov Y. **oral presentation**
- Philippine Society of Soil Science and Technology (PSSST) 2016: “*Land-use intensification affects phosphorus fractions in highly weathered tropical soils*”. Maranguit D.S., Guillaume T., Kuzyakov Y. **oral presentation**
- SPP 1685 & Ecosystem Nutrition 2015: “*Land-use impact on phosphorus fractions in tropical soil in Sumatra Indonesia*”. Maranguit D.S., Guillaume T., Kuzyakov Y. **oral presentation**

**Eidesstattliche Erklärung**

Hiermit erkläre ich eidesstattlich, dass die vorliegende digitale Kopie (.pdf) meiner Dissertation inhaltlich mit dem abgegebenen Prüfungsexemplar übereinstimmt



27.06.2017

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DeeJay Maranguit