Land use and biodiversity effects on P-transformation in soil

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List of Abbreviations

*	significance level $P < 0.05$
**	significance level $P < 0.01$
***	significance level $P < 0.001$
$a - c$	proportion of equilibrium fractionation
a	biological oxygen incorporation factor
a.s.l.	above sea level
AcidPase	potential acid phosphomonoesterase
AEG	grassland plot in ALB
Ag_3PO_4	silver phosphate
ANOVA	analysis of variance
Al	aluminium
ALB	Schwäbische Alb
APase	potential alkaline phosphomonoesterase
ATP	adenosine triphosphate
Bartlett *P*	Bartlett's Test of Sphericity
BExIS	Biodiversity Exploratories Information System
BG	background
C	carbon
c	proportion of kinetic fractionation
Ca	calcium
Cmic	microbial bound carbon
Corg	organic carbon
CV	coefficient of variation
d	day
df	degrees of freedom
e. g.	exempli gratia, for example
Equ.	equation
ε	isotopic fractionation factor
Fe	iron
g	gram
h	hour
H	hydrogen
H	treatment with high labelling ($\delta^{18}O_{SW}$ = +100 ‰)
H_2O_{dest}	distilled water
H_2SO_4-P	occluded phosphorus
HAI	Hainich-Dün
HCl-P	stable phosphorus
i. e.	id est, that is
kg	kilogram
KMO	*Kaiser-Meyer-Olkin* index
L	treatment with low labelling ($\delta^{18}O_{SW}$ = -33 ‰)
m	meter
M	treatment with medium labelling ($\delta^{18}O_{SW}$ = +50 ‰)
mg	milligram
Mg^{2+}	magnesium
min	minute
MIP	middle intensive (sampled) plots
mm	millimeter
N	nitrogen
n	sample size
na	not available
$NaHCO_3$-P	labile phosphorus
NaOH-P	moderately labile phosphorus
Nmic	microbial bound nitrogen
O	oxygen
O_{SW}	oxygen from ambient water

P	phosphorus
P	significance level
PCA	principal component analysis
Phex	hexanol extracted phosphorus
Pi	inorganic phosphorus
Pmic	microbial bound phosphorus
p-NP	*p*-nitrophenol
Po	organic phosphorus
PO_4	phosphate
PPase	potential pyrophosphatase
R	organyl group
r	*Pearsons*'s rank correlation coefficient
resin-P	phosphorus in solution
RMSEA	root mean square error of approximation
SCH	Schorfheide-Chorin
sd	standard deviation
se	standard error
SEM	structural equation model
Σ	sum
T	temperature
t	time of incubation
u. a.	unter anderem
VIP	very intensive (sampled) plots
vs.	versus
VSMOW	Vienna Standard Mean Ocean Water
$\delta^{18}O_{Pi}$	$\delta^{18}O$ of resin-P
$\delta^{18}O_{Pi/Equ}$	$\delta^{18}O$ of resin-P released via equilibrium reactions
$\delta^{18}O_{Pi/kin}$	$\delta^{18}O$ of resin-P released via kinetic reactions
$\delta^{18}O_{Po}$	$\delta^{18}O$ of organic phosphorus
$\delta^{18}O_{SW}$	$\delta^{18}O$ in soil water

Zusammenfassung

Für alle Lebensformen stellt Phosphor (P) neben Stickstoff (N) einen essentiellen Nährstoff dar. Im Gegensatz zu N kann P allerdings nicht über die Atmosphäre zurückgewonnen werden. In Hinblick auf vorhergesagte Erschöpfung der P-Ressourcen, sind die Kenntnisse der P-Transformationsprozesse im Boden daher von entscheidender Bedeutung.

Die Bedeutung von organisch-gebundenem P (Po) ist sehr gut erforscht, aber immer noch ist die quantitative Stellung von mikrobiell gebundenem P (Pmic) im Boden innerhalb des P-Kreislaufs unklar. Mikrobielle Biomasse im Boden immobilisiert P zwar, ist aber gleichzeitig eine wichtige Nährstoffquelle durch die hohe Umsatzrate. Daher war das Ziele dieser Studie, herauszufinden, ob die abiotischen und biotischen Variablen im Boden, welche Pmic kontrollieren, im Grünland und Wald gleich sind. Außerdem sollte der Effekt von Region (Standort) und Landnutzung (Grünland: Düngung, Mahd, Beweidung; Wald: Baumbestand, Forstmanagement) untersucht werden. Dafür wurden in drei Regionen in Deutschland, in der Schwäbischen Alb (ALB), im Hainich-Dün (HAI) und in der Schorfheide-Chorin (SCH), jeweils 150 Wald- und 150 Grünlandflächen, die sich in Pflanzendiversität und Landnutzung unterscheiden, beprobt. Mittels Structural Equation Modellierung konnte organisch-gebundener Kohlenstoff als fundierter Treiber für Pmic im Grünland herauskristallisiert werden. Regionale Unterschiede für Pmic bedingt durch unterschiedliche Umweltbedingungen (u. a. pH, Bodenfeuchte) konnten sowohl für Wald- als auch für Grünlandflächen gemessen werden. Analysiert man die drei Regionen jedoch separat, haben auf den Waldflächen weder Baumbestand noch Forstmanagement (bewirtschaftet vs. naturbelassen) einen signifikanten Einfluss auf Pmic im Boden. Obwohl 2014 bei einer wiederholten Messung von Pmic Unterschiede in der Grünlandnutzung bei Mahdanzahl (im HAI) und Landnutzung (in SCH) berechnet wurden, scheint Pmic im Grünland eher unempfindlich gegenüber der Landnutzung (Düngung, Beweidung, Mahd). Auch in Abhängigkeit des Bodentyps wurden mit Ausnahme des Histosols keine Unterschiede gefunden. Demzufolge neigen zwar die Einflussvariablen auf Pmic Varianzen aufzuzeigen, regionale Unterschiede der Einflussvariablen scheinen aber einen größeren Einfluss auf Pmic im Boden zu haben als jene, die aufgrund von Landnutzungsmanagement entstehen.

Die wichtigste Form des P ist das *ortho*-Phosphat (Pi), da es direkt von Mikroorganismen und Pflanzen aufgenommen werden kann. Im Boden aber liegt Po anteilig von 30 – 50 % des Gesamt-P vor.

Um Defizite der P-Limitierung zu kompensieren haben Pflanzen und Mikroorganismen Strategien entwickelt. Eine Möglichkeit ist Pi-Freisetzung via Gleichgewichtsfraktionierung. Hierbei erfolgt in Abhängigkeit der Temperatur ein intra-zellulärer Austausch aller vier Sauerstoff-(O)-Atome zwischen Phosphat und Wasser – die Pyrophosphatase. Die zweite Möglichkeit ist eine kinetische

Fraktionierung. Hierbei erfolgt der Austausch extra-zellulär. Speziellen Enzyme, sogenannte Phosphatasen, spalten durch Hydrolyse von Phosphorsäureestern pflanzenverfügbares Pi ab. Während dieser enzymatischen Hydrolyse von Po und Pyrophosphat werden O-Atome des Umgebungswassers in das freigesetzte Pi eingebaut. In einem Laborversuch konnte dieser Austausch an naturnahem Boden (ohne Zusatz von Substraten) nachgewiesen werden. Hierfür wurde ein Boden aus der ALB mit Wasser unterschiedlicher O-Isotopie ($\delta^{18}O_{SW}$) inkubiert und mittels einer Regression zwischen der O-Isotopie des gewonnenen Pi ($\delta^{18}O_{Pi}$) und der $\delta^{18}O_{SW}$ der biologische Einbau von O zu $\delta^{18}O_{Pi}$ während der Inkubation quantifiziert. Dabei dominierte die enzymatische Hydrolyse mit der Gleichgewichtsfraktionierung (> 90 % des gesamten biologischen Einbaus) gegenüber der kinetischen Fraktionierung. Der Einbau von O-Atomen aus dem Umgebungswasser in das Pi ist enzymgesteuert und somit von der Enzymaktivität abhängig. Normalerweise ist über Substratapplikationen nur die potentielle Enzymaktivität messbar. Durch den Einsatz von markiertem Wasser mit ^{18}O lassen sich durch Messung aber auch Rückschlusse auf die aktuelle Enzymaktivität ziehen.

Summary

Phosphorus (P) is next to nitrogen (N) an essential nutrient for all living organisms. In contrast to N from volatile species are known, P cannot be replenished from the atmosphere. Phosphorus is primarily derived from mineral deposits, which will be depleted in the future. Therefore, a better management of P cycle is mandatory.

Soil microbial biomass not only immobilizes P, but itself is an important source of nutrients because of its fast turnover. The importance of organic P (Po) is well-known, but still the quantitative significance of soil microbial P (Pmic) in the P cycle is rather unclear. Thus, the objectives were to elucidate whether the abiotic and biotic variables controlling Pmic concentrations in soil are the same for forests and grasslands. Moreover, to assess the effect of region and land-use management on Pmic concentrations in forest and grassland soils as mediated by the controlling variables. In three regions of Germany, Schwäbische Alb (ALB), Hanich-Dün (HAI) and Schorfheide-Chorin (SCH), forest and grassland plots (each n = 150) were studied differing in plant diversity and land-use management. Using structural equation models, organic carbon is the profound driver of plant diversity effects on Pmic in grasslands. For both forest and grassland, regional differences in Pmic attributable to differing environmental conditions (pH, soil moisture) were found. Analysing the three regions separately, neither tree species identity nor management (managed vs. near-natural) had significant effects on Pmic concentrations in forest soils. Although, for 2014 differences in cutting (HAI) and land-use (SCH) were found, in grassland soil, generally, Pmic seems to be insensitive to management (fertilization or harvesting). Also, there are no differences between soil type, excepted Histotols in SCH. Consequently, variables controlling Pmic in soil tend to show variance and regional alterations in controlling variables are more important for Pmic in soil than those induced by management.

From plants and microbes in natural environments the preferred source of P is inorganic ortho-phosphate. Organic P contributes to 30 – 50 % of total P concentrations. In order to overcome the deficiency of P limitation, plants and microorganisms developed strategies to contribute to the transformation of Po to Pi by synthesizing enzymes capable of hydrolysing Po. During the enzymatic hydrolysis of Po and of pyrophosphate, oxygen (O) atoms from ambient water are incorporated into the released Pi. Incubating one soil sample of the ALB with waters differing in the O isotope composition ($\delta^{18}O_{SW}$) in laboratory and using a linear regression of released Pi ($\delta^{18}O_{Pi}$) values on $\delta^{18}O_{SW}$ values in soil water the total biological O incorporation into Pi during the incubation could be quantified. Enzymatic hydrolysis associated with equilibrium fractionation dominated (> 90 % of the total biological O incorporation) in contrast to kinetic isotopic fractionation. Hence, this allowed to perform measurements of actual enzyme activity in contrast to conventional potential enzyme activity.

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1 Introduction

In general

Phosphorus (P) is a basic component of some of the most important biological moleculescommon to all organisms. For example, it is part of DNA and RNA, as well as the principal biochemical energy carrier (adenosine triphosphate – ATP) (Westheimer 1987). Therefore, P is an essential nutrient for all living organisms, which will be transferred from soil to animals via plants and is stored in bones or teeth (Plante 2007). Next to nitrogen (N), P is one of the most limiting macronutrients for all living organisms in both aquatic and terrestrial ecosystems (Turner et al. 2004, Vitousek et al. 2010). In recent years, it has become apparent that P limitation of primary producers and soil microorganisms is much more widespread in terrestrial ecosystems than previously thought (Elser et al. 2007, Vitousek et al. 2010). Anthropogenic N inputs due to combustion of fossil fuels and N-based fertilizers may further shift terrestrial systems towards P limitation (Holland et al. 2005). Under such a scenario, knowledge of processes contributing to P release in soil, and thus biological P nutrition, is required to evaluate potential consequences for P cycling.

In contrast to N, that can be atmospherically derived, P cannot be replenished except from the atmosphere once it is removed with the harvest or via erosion (Stevenson and Cole 1999). Consequently, P is primarily rock derived and will be depleted in future (Walker and Syers 1976, Gilbert 2009, Plante 2007). Resulting from the lack of economically feasible P deposits (**Figure 1.1**), a better management for P cycle is mandatory (Bünemann et al. 2011). Nevertheless, many processes of the P cycle are still understood matter of debate (Illmer and Schinner 1992) since discovering of P by Henning Brandt in 1669 (Bertrand 1994). The limiting effect and the limited deposits, thus, justify terming P as the “bottleneck of the world hunger” (Rorty 1946).

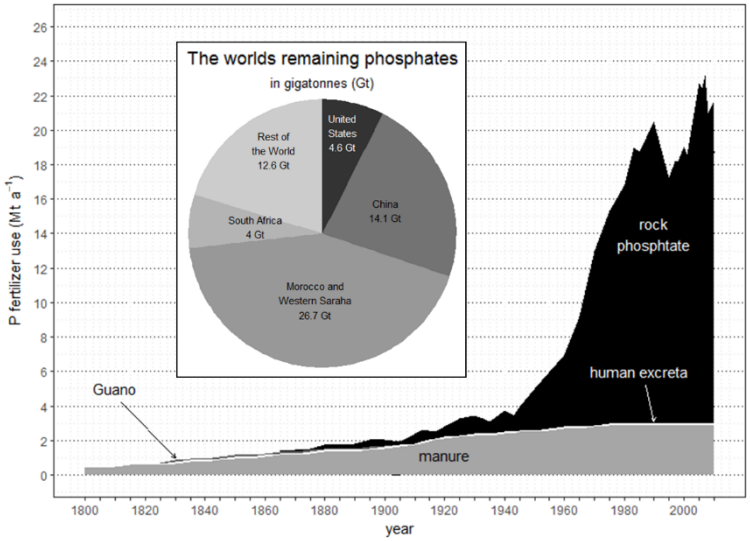

Figure 1.1: Historical sources of P fertilizers used in agriculture globally (1800 – 2010) in megatonnes per year (Mt) (Ashley et al. 2011) in comparison to the worlds remaining phosphates in gigatonnes (Gt) (Gilbert 2009).

Phosphorus is present in soil in various inorganic (Pi) and organic (Po) species (Hedley et al. 1982, Turner et al. 2004) and occurs in various forms: $H_2PO_4^-/HPO_4^{2-}$ ions in soil solution and adsorbed to mineral surfaces (labile: $NaHCO_3$-P), P more strongly sorbed to iron and aluminium oxides (moderately labile: NaOH-P), P bound in calcium phosphates (stable: HCl-P) and occluded P, e. g. spatially separated from P transformation reactions in secondary minerals such as pedogenic oxides (occluded: H_2SO_4-P) (Cross and Schlesinger 1995, Hedley et al. 1982, Negassa and Leinweber 2009). In most soils 30 – 50 % of the total P is incorporated into organic molecules. However, Po contents of various ecosystems have been demonstrated to range from as low as 5 % to 95 % (Plante 2007). Despite the importance of Po, its chemical nature has not yet been fully characterized (Plante 2007). Complex biotic and abiotic processes, Pi and Po species present in soil, and the fact that P only has one stable isotope hamper the direct traceability of P in soil.

Microbial biomass

Microbial biomass, which is composed of bacteria, fungi and other microbiota, is a major controlling factor of nutrient transformation and -cycling in soils (Bliss et al. 2004).On the one hand, P is incorporated into living microbial biomass and thus, is being immobilized (Achat et al. 2010a, 2010b, Oberson and Joner 2005), on the other hand, soil microbial biomass not only immobilizes P but itself is an important source of nutrients because of its fast turnover (Jenkinson and Ladd 1981, Stewart and Tiessen 1987). The P content in microbial biomass can vary widely depending on the age of organisms, the growth conditions and the P content of the growth medium (Tate 1984).

Previous studies on ecosystem development have related the importance of total P to plant biomass (Bieleski 1973, Wardle et al. 2004). Also, the importance of Po was increasingly recognised (Turner et al. 2005, Turner et al. 2007, Walker and Syers 1976). Still, the quantitative significant role of soil microbial P (Pmic) in the P cycle lacks scientific attention (Chen et al. 2000, Jenkinson and Ladd 1981, Oberson and Joner 2005, Richardson and Simpson 2011, Turner et al. 2013). As the turnover of Po depends on the activity of the microbial biomass (Guggenberger et al. 1996), it is key for mineralisation of Po in soil (Brookes et al. 1984). Boundary conditions are known to influence the microbial biomass concentration in soil, e. g. via soil moisture (Chen et al. 2003), soil pH (Dou and Steffens 1992) or plant diversity (Grayston et al. 2001).

However, the understanding of environmental conditions as drivers of Pmic in soil is still in its infancy. Only a few variables thought to control Pmic in soil have been included in the studies published so far, which, however, often failed to consider the impact of plants. Joergensen et al. (1995) and Stutter et al. (2015) correlated organic carbon (Corg) concentrations to Pmic concentrations in forest and grassland soils. Constraining the growth of microorganisms (Wardle 1992), their results probably reflected the role of Corg as an ultimate energy source for heterotrophic soil organisms. Liebisch et al. (2014) found increasing Pmic concentrations related to increasing soil moisture in a grassland fertilization experiment. These results are in line with a positive effect of soil moisture on microbial biomass carbon (Cmic) concentrations in soil demonstrated in a meta-analysis performed by (Wardle 1998). Moreover, Liebisch et al. (2014) found highest Pmic concentrations in soils with moderately high pH (6.8), which might result from the strong pH control of the $H_2PO_4^-/HPO_4^{2-}$ ion concentrations in soil solution, that are serving as the pool of P readily available for uptake by soil microorganisms. Total P concentrations in soil have been shown to be decisive for Pmic concentrations in forest soils under beech (Joergensen et al. 1995). As microbes are an integral part of the P cycle, they are likely to be linked to other P fractions in soil (Tate 1984, Tate et al. 1991, Khan and Joergensen 2012), because microbes can act as a source and sink of P while turning over Po and Pi fractions in soil (Tate 1984, Brookes 2001, Griffiths et al. 2012, Burns et al. 2013). Additionally, plants have been demonstrated to control all of these variables, except total P concentration, in an ecosystem. Therefore, plants definitely are also exerting an effect on Pmic in soil. In artificial grasslands and established forests, it has been shown that not only the mere existence or quantity of plants biomass is key to Corg stocks, but also their diversity positively correlates to increased Corg stocks, which likely results from increased above- and belowground litter input (Fornara and Tilman 2008, Steinbeiss et al. 2008, Gamfeldt et al. 2013, Lange et al. 2015).

In this study Pmic concentrations were assumed to vary between regions with different climatic conditions, e. g. precipitation controlling soil moisture and other factors, such as parent rock material, soil pH, P fractions in soil, Corg, and plant diversity. Regional differences might further be enhanced by

land use and land management practices. For example, the management of forests is controlled by the selection of mixtures of tree species or monocultures and their varying harvest intervals. Various tree species are known to influence soil properties such as pH and C : N ratios and are thereby potentially affecting Pmic concentrations in soil. Because tree species might as well have been planted according to their ecological amplitude, e. g. coniferous trees at sites with low soil pH, cause and effect are difficult to disentangle in established, non-natural ecosystems. Nevertheless, a superior correlation between different environmental factors and the P cycle should remain valid besides the effects of tree species. To the best of the knowledge, only Aponte et al. (2013) have been studying effects of tree species on Pmic concentrations in soil. In this study, two Mediterranean oak species resulted in different Pmic concentrations in soil.

In grasslands, management affects Pmic concentrations in soil via fertilizer inputs, which are likely associated with indirect effects, such as changes of soil pH and availability of P. On agricultural soils, commonly inorganic P-fertilizers (produced from rock phosphates), manure or composts (Ayaga et al. 2006) are used. Common to these fertilisers is an increased substrate availability for microorganisms. Grasslands are moreover managed via grazing. Reduced grazing (and fertilization) intensity has been shown to increase microbially-mediated processes resulting in microbial immobilization of scarce nutrients including P (Stutter et al. 2015). Therefore, increased Pmic concentrations can be expected at sites with a low management intensity. But the opposite might also be true, i. e. high management intensity in terms of grazing and fertilization associated with high Pmic concentrations in soil. As reasoned above, this might be caused by positive effects of fertilization combined with the ability of microorganisms to accumulate P as polyphosphates in situations of an excess P supply (Condron et al. 2005).

P uptake

Inorganic ortho-phosphate (Pi) is the preferred source of P taken up directly by microorganisms and plants in natural environments, (Liang and Blake 2006b). In contrast to Pi, organically bound P (Po) is not directly available to organisms, because it cannot be adsorbed into cells (Plante 2007). Plants take up P as dissolved P (H_2PO4^-) from the soil solution (Smith 2002, Smith et al. 2003, Rae et al. 2003). For cellular uptake to occur, P must first be released from organic molecules via mineralisation. The final stage of the conversion of Po to Pi occurs through the enzymatic activity of, the so called phosphatases (Plante 2007).

P cycle

Several pathways overcome P limitation by making P available as Pi. Dissolved Pi can either form from the dissolution of phosphate (PO_4) containing minerals such as apatites (geochemical pathway) or Pi is

released from Po (biochemical pathway) (Plante 2007). The geochemical cycle is linked intimately to biological activity and many diagenetic reactions involving PO_4 are mediating by living organisms (Blake et al. 1997, Paul and Clark 1996). The biochemical pathway is defined in terms of biological constituents or stability and the transformations are primarily microbial mediated (Plante 2007), as microorganisms and plants have developed the ability to exudate phosphatase enzymes (Richardson et al. 2011). Generally disentangling the two subcycles (geochemical and biochemical) within the P cycle is difficult. Resulting from the low solubility product of PO_4 (Fassbender et al. 1966), biological mediated PO_4 precipitates as novel (bio-)mineral phases or sorbed on sesquioxide surfaces (Chen et al. 2010). By means of the currently used extraction protocols, extracted PO_4 will exclusively be assigned to geochemical processes, although it may have been formed biogenically. The other part of the biochemical subcycle is the hydrolysis of Po, known to occur via exoenzymes (Blake et al. 2005). This processis associated with a kinetic fractionation while oxygen (O) atom(s) are exchanged in the newly built PO_4 with O atom(s) from ambient water (Liang and Blake 2006b, a). Therefore, $P^{18}O_4$ could be used as tracer when studying the biochemical cycling of P (Blake et al. 2005).

Enzymes

Conditions of limited Pi initiate a series of genetically controlled reactions, which allows microorganisms to utilize more recalcitrant Po compounds as a source of Pi for their proliferation. In order for organisms to utilize most Po compounds, specialized enzymes, so called phosphate-saving enzymes, must be expressed and released into the interstitial space to break down large and complex Po molecules into smaller units and release organically bound PO_4 as free Pi (Blake et al. 2005).

It is generally assumed that Po cannot be taken up directly by plants and microorganisms because of the lack of transport shuttles through the cell membrane. Instead organisms must take up P as dissolved Pi (H_2PO4^-) from the soil solution (Rae et al. 2003, Smith 2002, 2003). Therefore, organisms developed strategies to contribute to the transformation of Po to Pi, e. g. by the expression of enzymes capable of hydrolyzing ester-bonds in Po (Richardson and Simpson 2011). These enzymes are termed “phosphatases”, and embrace a diverse group of enzymes, which catalyze hydrolysis of Po (Nannipieri et al. 2011, Schmidt and Laskowski 1961). 70 – 80 % of the microbial population, including bacteria and fungi, has been shown to express phosphatases (Plante 2007).

Enzymes are categorised according to what they do, rather than what they are (Florkin and Stotz 1965, Corbridge 1990). Enzymes catalysing hydrolysis, are known as hydrolases and if the compounds they are acting upon (substrates) are esters they are known as esterases. Enzymes specific to phosphate esters are known as phosphoesterases or phosphatases (Corbridge 1990).

The commission on enzymes of the International Union of Biochemistry has classified all these enzymes into five major groups (Florkin and Stotz 1965). These include the phosphoric monoester

hydrolases, phosphoric diester hydrolases, triphosphoric monoester hydrolases, enzymes acting on phosphoryl-containing anhydrides and enzymes acting on P-N bonds, such as phosphoamidases (Tabatabai 1994). Various studies have shown that acid phosphatase is predominant in acid soils and alkaline phosphatase in alkaline soils (Eivazi and Tabatabai 1977, Juma and Tabatabai 1978, Tabatabai 1994). Hence, the pH at which the phosphatases are most effective determines their categorisation into alkaline or acid phosphatases (Corbridge 1990).

Commonly, enzyme activity assays are based on substrate addition and therefore represent the potential enzyme activity under optimized conditions (Eivazi and Tabatabai 1977, Tabatabai and Bremner 1969), whereas appropriate methods measuring the actual enzyme activity in natural systems are lacking (Nannipieri et al. 2011). During the enzymatic hydrolysis of Po and of pyrophosphate, O atoms from ambient water are incorporated into the released PO_4 (Blake et al. 1997, 2005, Gross and Angert 2015, Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015). In contrast, there is no or negligible exchange between O atoms in ambient water and dissolved Pi during geochemical Pi release, i. e. dissolution or desorption (Jaisi et al. 2010, Liang and Blake 2007). Therefore, enzymatically released Pi can be targeted unambiguously if isotopically labeled water is incorporated into released Pi during incubation.

Cohn (1949) performed the first study demonstrating that during the enzymatic hydrolysis of phosphomonoester one O atom from ambient water (O_{SW}) was incorporated into the released PO_4. Organic P is degraded catalysed by enzymes in one (monoester) or two (diester) steps (Liang and Blake 2009). The first step is the hydrolytic cleavage of phosphodiester bonds that results in the generation of phosphomonoesters (**Figure 1.2**a) and the second step is the conversion of phosphomonoesters to organic groups and the release of Pi (**Figure 1.2**b) (Liang and Blake 2009).

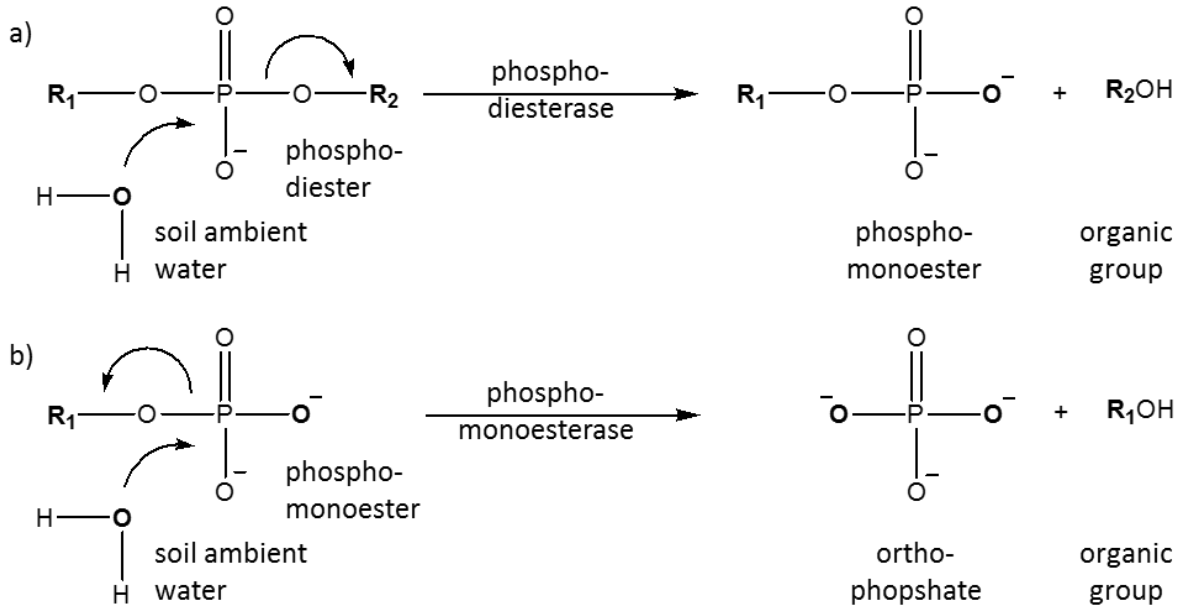

Figure 1.2: Two-step bond cleavage of phosphodiesters to release free PO_4. a) Step 1: cleavage of phosphodiester bond and formation of phosphomonoester, and b) Step 2: cleavage of phosphomonoester bond and release of free PO_4 after Liang and Blake (2009) (R – organyl group).

Released Pi from Po by extra-cellular phosphatases differs in its O isotope composition in comparison to Pi which is in an equilibrium with the cell water (Liang and Blake 2006b, 2009). The incorporation induces an isotopic fractionation, which can be described by an isotopic fractionation factor (ε). Studies demonstrated that enzymes exposed to various substrates show alternating ε, which is thought to be associated with monoesterases or diesterases being active (Liang and Blake 2009). For monoesterases a substrate induced effect on ε **(Equ. 1.1)** was shown by von Sperber et al. (2014).

$$\delta^{18}O_{Pi/monoesterase} = 0.25 \cdot (\delta^{18}O_{SW} + \varepsilon) + 0.75 \cdot \delta^{18}O_{Po} \quad \text{(Equ. 1.1)}$$

Liang and Blake (2009) found that the diesterases are not compound specific in terms of substrate they are hydrolysing, but by means of the O isotope effect it was demonstrated that the hydrolysis reaction is substrate specific. If diester and monoester are occurring simultaneously, the ε is the combined fractionation factor associated with breaking diester and monoester bonds **(Equ. 1.2)** (Liang and Blake 2009).

$$\delta^{18}O_{Pi/diesterase} = 0.5 \cdot \delta^{18}O_{Po} + 0.25 \cdot (\delta^{18}O_{SW} + \varepsilon_1) + 0.25 \cdot (\delta^{18}O_{SW} + \varepsilon_2) \quad \text{(Equ. 1.2)}$$

In both, model systems and in soil, the proportion of O incorporation into the released Pi depends upon the number of ester bonds to be broken, e. g. 25 % of O atoms incorporated from ambient water

into Pi released from monoester-Po and 50 % in the case of the cleavage of diester-bond Po (Liang and Blake 2006b, 2009, von Sperber et al. 2014).

Special hydrolases are inorganic pyrophosphatases (PPase). PPase are intra-cellular enzymes (Knowles 1980) which catalyse the reversible hydrolysis of the high-energy bond in inorganic pyrophosphates (**Figure 1.3**) formed as the product of the many biosynthetic reactions that utilize ATP, known to drive thermodynamically unfavourable reactions in the cell (Chang and Blake 2015), in presence of magnesium (Mg^{2+}, Kunitz and Robbins (1961)).

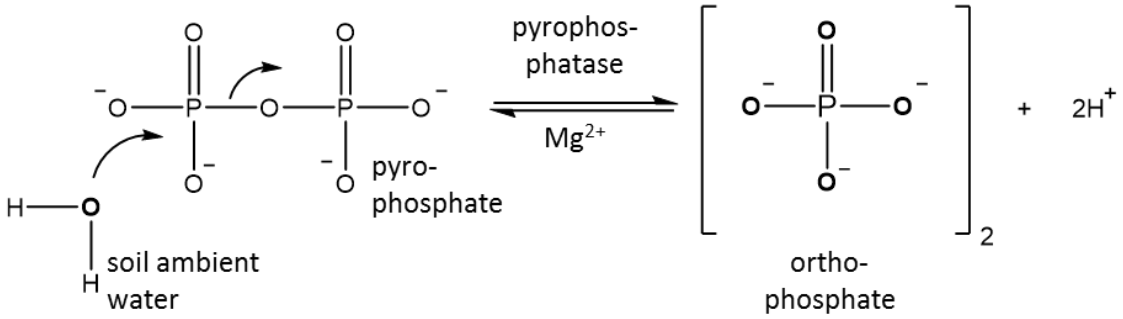

Figure 1.3: Hydrolysis of pyrophosphate to phosphate catalysed by pyrophosphatase in presence of magnesium (Mg^{2+}) after Kunitz and Robbins (1961).

On the one hand this reversible reaction is necessary to control the level of dissolved pyrophosphate in cells, because if pyrophosphate increases in the cells growth stops (Chen et al. 1990). On the other hand, low pyrophosphate levels hamper synthesis of important biomolecules such as RNA or DNA (Chang and Blake 2015, Klemme 1976). During this reaction an exchange of all four O atoms of PO_4 (**Figure 1.3**) in dependence of temperature (*T* in Kelvin) occurs, calculated by Longinelli and Nuti (1973) modified by Chang and Blake (2015) **(Equ. 1.3)**:

$$\delta^{18}O_{Pi/PPase} = e^{\left(\frac{14.43}{T} - \frac{26.54}{1000}\right)} \cdot [\delta^{18}O_W + 1000] - 1000 \quad \text{(Equ. 1.3)}$$

The incorporation of O atoms from ambient water into enzymatically released Pi was studied by means of incubation of bacteria, enzymes, or Po substrates with waters differing in their isotopic composition of oxygen ($\delta^{18}O_{SW}$) (Blake et al. 1997, 2005, Gross and Angert 2015, Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015). However, these studies applied model organisms and specific substrates, which is rendering their relevance for in-situ conditions in soil with a multitude of microorganisms, enzymes, and Po species questionable.

Usually, with substrate application, the potential enzyme activity is verifiable (monoesterase: Tabatabai and Bremner (1969), diesterase: (Browman and Tabatabai 1978), triesterase: (Eivazi and

Tabatabai 1977), PPase: (Dick and Tabatabai 1978)). Therefore, the kinetics of the incorporation of ^{18}O labelled water into released PO_4 might serve as tool for assessing actual enzyme activity.

While esterase enzymes are exuded by microorganisms, PPase mainly acts on pyrophosphate stored inside the cells of organisms (Burns et al. 2013). During the transformation processes of Pi in the cytoplasm and upon the action of PPase, successively all four O atoms are being exchanged by O atoms from ambient water (Blake et al. 2005, Chang and Blake 2015, Longinelli and Nuti 1973). In addition, isotopic fractionation accompanying O incorporation during enzymatic Pi release must be considered. In contrast to kinetic isotope fractionation induced by extra-cellular enzymes (Liang and Blake 2006b, 2009, von Sperber et al. 2014), PPase activity is associated with a temperature-dependent equilibrium fractionation (Blake et al. 2005, Chang and Blake 2015, Longinelli and Nuti 1973, Cohn 1953). The above-mentioned laboratory studies by Liang and Blake (2006b, 2009) and von Sperber et al. (2014) used specific substrates and enzymes, which could be assigned to either kinetic or equilibrium fractionation, and calculated the proportion of O incorporation from isotopically labeled, ambient water. Given the mixture of substrate compounds and enzymes in soil, it is impossible to derive hydrolytic pathways of Po transformations. Nevertheless, the differentiation between kinetic or equilibrium fractionation is possible if appropriate calculations are used. In contrast to the addition of isotopically labeled water as described above, isotopically labeled Pi ($\delta^{18}O_{Pi}$) or Po ($\delta^{18}O_{Po}$) has been used as well to yield insights into transformation processes involved in P cycling (Gross and Angert 2015, Gross et al. 2015, Joshi et al. 2016, Melby et al. 2013). However, the addition of P sources – be it inorganic or organic – potentially affects the composition of the microbial community and its activity in soil. Therefore, the addition of isotopically labeled water is advantageous over isotopically labeled P-containing substrate additions, if the P cycle is to be studied under natural conditions. After the addition of isotopically labeled water and a certain incubation time (ti), the O isotope signature of Pi present in soil solution ($\delta^{18}O_{Pi/ti}$) comprises contributions of i) PPase activity releasing Pi carrying the isotopic signature of equilibrium fractionation ($\delta^{18}O_{Pi/Equ}$, fraction *a* – *c*, **Figure 1.4**), and of ii) Pi released by phosphatases associated with kinetic fractionation (with $\delta^{18}O_{Pi/kin}$, fraction *c*, **Figure 1.4**) in conjunction with iii) a pool that is not influenced by enzymatic activity (either present before labeling or released by abiotic processes, non-labeled background $\delta^{18}O_{Pi/t0}$ values, fraction 1 – *a*, **Figure 1.4**):

$$\delta^{18}O_{Pi/ti} = (a - c) \cdot \delta^{18}O_{Pi/Equ} + c \cdot \delta^{18}O_{Pi/kin} + (1 - a) \cdot \delta^{18}O_{Pi/t0} \quad \text{(Equ. 1.4)}$$

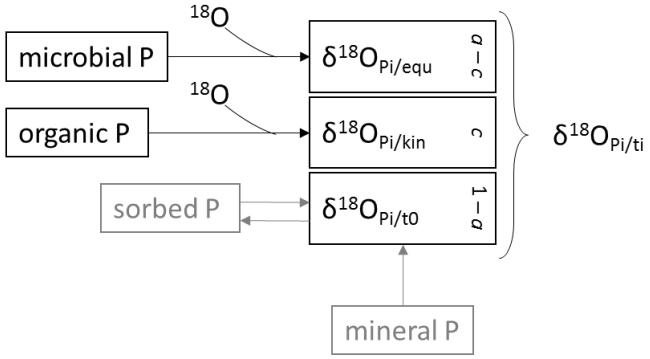

Figure 1.4: Conceptual sketch of a three end-member mixing model illustrating biological O incorporation into inorganic P (Pi) released during incubation of a soil sample with isotopically labeled water ($H_2{}^{18}O$ as an example). After incubation, Pi present in soil solution (captured as resin-extractable Pi with $\delta^{18}O_{Pi/ti}$ as the O isotopic signature in phosphate) could originate from three sources: i) Pi released from microbial cells (actively or by cell lysis) associated with the incorporation of ^{18}O atoms from ambient (= labeled) water by thermodynamic equilibrium fractionation yielding $\delta^{18}O_{Pi/equ}$ values that can be calculated using temperature and $\delta^{18}O$ values of soil water (Chang and Blake 2015), ii) Pi released during the hydrolysis of Po, e. g. bound as esters, associated with the incorporation of ^{18}O atoms from labeled water by kinetic fractionation and thus, by enzyme- and substrate-specific kinetic fractionation factors (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015), and iii) phosphate molecules that had been present already before incubation reflecting the initial O isotopic signature in phosphate ($\delta^{18}O_{Pi/t0}$). Assuming steady state conditions during incubation, this source contains desorption and dissolution processes known for no/negligible exchange between O atoms in ambient (= labeled) water and in phosphate (Jaisi et al. 2010, Liang and Blake 2007). Each source can be characterized by its fractional contribution ($a - c$, c, $1 - a$) to Pi present in soil solution after incubation.

Research needs

Former Pmic studies were lacking information on the comparison of grassland and forest soils at same study site under different land use and management. Whereas previous studies focused on a smaller set of land-use types and environmental factors driving Pmic storage in microorganisms, this study is to be the first to use a larger data basis as required for generalizing these results and potentially uncover environmental conditions controlling Pmic in soils, that have not yet been accounted for in the literature (C : N ratios and P fractions in soil). Recently developed statistical tools such as structural equation models (SEM) allow o separate direct from indirect effects in large data sets based on linear regressions (Grace et al. 2012). Former studies for O incorporation into released Pi added enzymes and/or known P sources. The addition of P sources potentially affects the composition of the microbial community and its activity in soil. Therefore, this study aimed at reducing bias in comparison to in-situ conditions by means of using untreated soils without sterilization, addition of different P sources or enzymes. By doing so it meant to reduce the ambiguity of using an empirical method to separate the contribution of equilibrium fractionation and kinetic fractionation on the measured isotope values in the soil system involved in the transformation of Po to Pi in soil.

Research questions and objectives

I addressed the following research questions in this thesis:

a) How do soil chemical properties, land use and plant diversity influence microbial phosphorus in forest and grassland soils? (**Section 3.1**)

b) Microbial phosphorus of grassland sites: Are there differences between years? (**Section 3.2**)

c) Is it possible to quantify the enzyme-mediated hydrolysis by means of the oxygen isotope ratio of phosphate in soil? (**Section 3.3**)

The main objectives of the Pmic study were (i) to elucidate whether abiotic and biotic variables controlling Pmic concentrations in soil are the same for forests and grasslands, and (ii) to assess the effect of region and management on Pmic concentrations in forest and grassland soils as mediated by the controlling variables. I hypothesized that (1) Corg concentrations, soil moisture as well as soil pH, soil C : N ratios, and P fractions control Pmic concentrations in soil with plant diversity exerting an additional effect in forests and grasslands, (2) regions with high annual precipitation, high Corg concentrations in soil, and high soil pH will be characterized by highest Pmic concentrations in soil (ALB > HAI > SCH), (3) in forests, Pmic concentrations in soil will decrease with increasing land-use intensity as high land-use intensity is associated with coniferous stands (low soil pH, high C : N ratios of litter), in grassland soils Pmic concentrations increase with increasing land-use intensity due to fertilization (**Section 3.1**).

In order to consider seasonal variations for Pmic, the investigations of Pmic were extended onto a study area becasue land-use management was not changed within three years. By means of data from the same season after three years, I aimed (iii) to investigate if Pmic levels are fluctuating between years and (iv) to extend Pmic investigations depending on soil type. Because of Corg is depending on soil type and Pmic is influenced by Corg I hypothesized (4) that there will be an effect of soil type on Pmic (**Section 3.2**).

Enzyme mediated PO_4 release was studied with the objective (v) to test whether O atoms from isotopically labelled water will be incorporated into PO_4 molecules released during incubation of soil samples. If so, I aimed at quantifying (vi) the contribution of the total biogenic O incorporation into the released Pi, which (vii) proportion the two processes in total biological O incorporation, i. e. equilibrium and kinetic isotope fractionation, have and (viii) the reliability of the underlying calculations and assumptions (**Section 3.3**).

2 Material and methods

2.1 Study sites

This study is part of the project "Biodiversity Exploratories" (www.biodiversity-exploratories.de) in which the influence of land-use type, land-use intensity, and biodiversity on ecosystem functioning is studied by an interdisciplinary research consortium (Fischer et al. 2010).

The project includes plots in three study regions in Germany: Schwäbische Alb (ALB), Hainich-Dün (HAI), and Schorfheide-Chorin (SCH) (**Figure 2.1**). Each region comprises 50 plots in grasslands and 50 plots in forests resulting in a total number of 300 plots. Plots in each region have been managed continuously by farmers and foresters for many years (Fischer et al. 2010). The oldest known land-use forms are meadows in ALB established in 1817 whereas in HAI and SCH arable land was converted to grassland in the 1980s and 1990s at the latest (personal communication Jan Thiele, University of Münster).

Figure 2.1: Study area (exploratories) located in Germany (Klaus et al. 2011).

The SCH is a young glacial landscape area of about 1300 km^2 size located in northeast Germany, Brandenburg. The HAI is located in central Germany, Thuringia. This area is affected by calcareous bedrocks and is about 1300 km^2 large. The ALB is located in southwest Germany, Baden-Württemberg. It is sized about 422 km^2 and characterized by the highest annual mean precipitation of these three study sites (Table **2.1**) (Fischer et al. 2010). Because of different geological and geomorphological background, the three exploratories are characterized by different soil types (Table **2.1**).

Table 2.1: Main geographical and environmental characteristics of this study regions (Fischer et al. 2010).

region	**Schwäbische Alb**	**Hainich-Dün**	**Schorfheide-Chorin**
abbreviation	ALB	HAI	SCH
located in Germany	south-west	central	north-east
altitude	460 – 860 m a.s.l.	258 – 550 m a.s.l.	3 – 140 m a.s.l.
mean temperature	6 – 7 °C	6.5 – 8 °C	8 – 8.5 °C
mean precipitation	700 – 1,000 mm	500 – 800 mm	500 – 600 mm
parent material	calcareous bedrocks with karst phenomena	calcareous bedrocks of Triassic limestone covered by loess	glacial till covered with glacio-fluvial or Aeolian sand
soil texture	rich in clay	loamy or clayey	sandy loam to pure sand
soil type	Cambisols, Leptosols	Cambisols, Stagnosols, Vertisols, Luvisols	Histosols, Luvisols, Gleysols, Albeluvisols, Cambisols, Regosols, Podzols
main tree species	beech, spruce	beech, spruce	beech, oak, pine
land-use type	*grassland*: meadow (*n* = 22), mown pasture (*n* = 9), pasture (*n* = 19) *forest*: age class forest (*n* = 46), unmanaged forest (*n* = 4)	*grassland*: meadow (*n* = 7), mown pasture (*n* = 23), pasture (*n* = 20) *forest*: age class forest (*n* = 28), unmanaged forest (*n* = 13), selection cutting (*n* = 9)	*grassland*: meadow (*n* = 18), mown pasture (*n* = 10), pasture (*n* = 22) *forest*: age class forest (*n* = 45), unmanaged forest (*n* = 5)

Each study site, forest as well as grassland site, comprises six „very intensive (sampled) plots“, so called VIP. For the *ex situ* incubation experiment soil samples from one VIP grassland plot in ALB (AEG) were taken: number six (AEG-06) located in south-west Germany. The study site AEG-06 is situated in the “Schwäbische Alb” at an altitude of 711 m a.s.l. and is characterized by a mean annual precipitation of 901 mm. **Table 2.2** provides the main geographical characteristics for AEG-06. Typically the soils of the ALB region are N limited (N : P < 10) (Klaus et al. 2011). The parent material is limestone (late Jurassic; Regierungspräsidium Freiburg Landesamt für Geologie Rohstoffe und Bergbau (2016)). Major physical and chemical soil properties are listed in **Table S 4 (Supplementary material)**.

Table 2.2: Main geographical characteristics of AEG-06.

sampling site	AEG-06
soil type [a]	Leptosol
geology [b]	part of the late Jurassic (late compact limestone); dominated by light grey up to light yellowish brown limestone with an unbedded structure, partly formed as coralgal limestone, partly marly, with off-reef facies and partly dolomitized or dedolomitizied
land use [c]	mown pasture
pH [d]	5.93

[a] Fischer et al. (2010), [b] Regierungspräsidium Freiburg Landesamt für Geologie Rohstoffe und Bergbau (2016), [c] Hailer and Ayasse (2016), [d] Schöning and Trumbore (2015)

2.2 Forest and grassland management

In both forests and grasslands, plots were selected to cover a wide range of land-use types typical for large parts of Central Europe. Forest plots are classified as unmanaged forest (*n* = 22), age class forest (*n* = 119), and selection cutting (*n* = 9). Age class forests have an even-aged stand structure due to harvests at 80 to 120-year intervals. Selection forests are uneven-aged deciduous stands, dominated by European beech (*Fagus sylvatica* (L.)), in which single or small groups of trees were harvested selectively. The forests in the ALB and HAI are dominated by European beech [*Fagus sylvatica* L.] (ALB: *n* = 38; HAI: *n* = 46) and Norway spruce [*Picea abies* (L.) Karst] (ALB: *n* = 12; HAI: *n* = 4). In the studied forests in SCH European beech (*n* = 21), sessile oak [*Quercus petraea* Liebl.] and/or English oak [*Quercus robur* L.] (*n* = 7), and Scots pine [*Pinus sylvestris* L.] (*n* = 22) are the dominating tree species (Schall and Ammer 2015).

Grassland types are grasses, herbs and legumes. The experimental plots are either fertilized (*n* = 63) or non-fertilized (*n* = 87) and further classified into the land-use types meadow (grasslands mown once to three-times per year, *n* = 47), mown pasture (grasslands mown and grazed within the same year, *n* = 42) and pasture (grasslands grazed by different livestock at different intensities, *n* = 61) (Lorenzen and Weisser 2016). The fertilization of P comprises application of organic fertilizers (e. g. liquid manure) and mineral-fertilizer application. Fertilizer application rates to fertilized grasslands during last vegetation period were 25 – 35 kg P ha^{-1}, 27 – 40 kg P ha^{-1} in ALB and SCH, respectively (Gockel and Weisser 2017). In addition to fertilization, differences in mowing frequency (number of cuts per year) and grazing intensity (livestock units × grazing days ha^{-1} $year^{-1}$) were included.

2.3 Soil sampling

For Pmic analysis soil samples were taken with soil corers (50 mm Ø, Eijelkamp, Giesenbeck, The Netherlands) in May 2011 (grassland and forest site) and May 2014 (grassland site). Samples were taken in a short time interval of three weeks to avoid possible P release from microbial biomass enhanced by drying-rewetting and trophic interactions of microflora and microfauna due to weather conditions (Cole et al. 1978). For each plot a mixed soil sample of 14 cores (0 – 10 cm soil depth) collected along two transects (40 m long in forests, 20 m long in grasslands) per plot was used. As a standard procedure for the coordinated soil sampling campaigns, organic layers were removed prior to mineral soil sampling at forest sites. As it will be aware that this procedure results in an underestimation of Pmic concentrations, but it facilitates the comparison between grasslands and forests as this study relied on mineral soil samples only. For the incubation experiment top-soil samples were taken from a depth of 10 cm in summer 2016 on a special grassland plot in ALB (AEG-06).
Immediately after sampling all soil samples were sieved (< 2 mm) to remove large pieces of gravel and roots to minimize the influence of living plant tissue (McLaughlin and Alston 1987) on enzyme activities (Helal and Sauerbeck 1984, Chen et al. 2004). In order to avoid quantitative and qualitative changes in microbial biomass, fresh soil samples (stored at 4 °C) were used for soil biological analyses. Soil chemical analyses were performed on air-dried soil samples.

2.4 Incubation experiment

After sieving, the water content of soil was measured (**Supplementary material**: **Table S 4**). An aliquot of 120 g field-fresh soil (soil: AEG-06) was filled in jars and the soil water content was adjusted to 38.0 ± 0.6 % by adding tap water. Samples were allowed to equilibrate for three days while keeping soil moisture constant. Thereafter, three different isotopically labeled waters (treatments low [L], medium [M], and high [H] with $\delta^{18}O$ values of water of -33, +50, and +100 ‰ VSMOW, respectively) were added resulting in a final water content of 41.0 ± 0.1 % (final mass per jar = 130 g) and adjusting the O values of soil water ($\delta^{18}O_{SW}$) to ^{18}O-depleted water (L: $\delta^{18}O_{SW}$ = -10.9 ± 0.1 ‰) and ^{18}O-enriched water (M: $\delta^{18}O_{SW}$ = +36.9 ± 0.8 ‰, H: $\delta^{18}O_{SW}$ = +59.2 ± 1.1 ‰), respectively.
The labeled soils were incubated in air-tight preserving jars to minimize the evaporation of water and associated isotopic enrichment effects (Luz et al. 2009). The preserving jars were stored at room temperature (20.2 to 21.4 °C) and incubated for different periods of time (ti = 1, 2, 4, 7, and 14 days) resulting in a total number of 45 jars (three treatments × five time steps × three replicates). In addition, three aliquots of non-labeled soil (ti = 0) serving as a background were analyzed as described below. After each incubation interval, three replicate jars were sacrificed.

2.5 Chemical analysis

For analysing soil water content, an aliquot of soil was dried to a constant weight at 105 °C in order to define a “dry” sample weight. The weight of the moist and dry soil was recorded and the percentage water per dry soil calculated (Gardner 1986).

In oder to determine Pmic, a combination of methods by Kouno et al. (1995) and McLaughlin et al. (1986) was used. The underlying principle is to lyse microbial cells of a field-fresh soil sample by hexanol fumigation, yielding the sum of P originating from microbial cells and of P extracted from the soil matrix. The latter pool can be subtracted from this sum by analysing P concentrations of a non-fumigated sample. Thus, the difference between the fumigated and non-fumigated sample represents P originating from lysed microbial cells. However, a proportion of P released during fumigation and extraction is removed from the solution by sorption and/or precipitation. To account for this loss, the difference between fumigated and non-fumigated samples must be corrected. This is achieved by a third treatment in which a known concentration of P is added to a non-fumigated sample (P spike). In all three treatments resin stripes (2 cm × 6 cm, anion exchange membranes, product code: 551642S VWR, Bruchsal, Germany) were used to extract P from the solution. Resin stripes were conditioned using 0.5 M $NaHCO_3$ (pH = 8.5). For each sample three aliquots of moist soil equalling 2 g of dry soil were weighed into 50 ml polyethylene tubes and 30 ml distilled water (H_2O_{dest}) was added to each tube. The first aliquot was fumigated with 1 ml liquid hexanol (fumigated aliquot). Only H_2O_{dest} was added to the second aliquot (non-fumigated aliquot) whereas in addition 1 ml of 20 µg P ml^{-1} (as dissolved KH_2PO_4) was added to the third aliquot (P spike). The samples were shaken horizontally for 16 hours with bicarbonate conducted resin membrane strips. Afterwards, the resin stripes were removed from the soils suspension, rinsed with H_2O_{dest} to remove adhering soil, and transferred to new tubes. Subsequently, 30 ml 0.1 M sodium chloride/hydrochloric acid was added, and the resin stripes were shaken again for two hours to desorb P. To correct for sorption of P released during fumigation in the calculation of hexanol P (Phex), a sorption curve was used resulting from the comparison of non-fumigated and P spiked aliquots (Bünemann et al. 2008). Phosphorus concentrations in the fumigated sample are a function of microbial P (Phex), which are (i) modified depending on the extent of sorption/precipitation (slope *a*), and (ii) shifted along the y axis depending on P extracted from the soil matrix in the non-fumigated aliquot (y axis intercept *b*; **(Equ. 2.1)**).

$$P\ (fumigated\ aliquot)\ = a \cdot microbial\ P\ (Phex) + b \qquad \text{(Equ. 2.1)}$$

(Equ. 2.1) can be rearranged to solve for microbial P (Phex) **(Equ. 2.2)**:

$$microbial\ P\ (Phex) = \frac{P\ (fumigated\ aliquot) - b}{a} \quad \text{(Equ. 2.2)}$$

The slope *a* is calculated as follows **(Equ. 2.3)**:

$$a = \frac{P\ (P\ spike) - P\ (non - fumigated\ aliquot)}{P_{mass\ (P\ spike)}} \quad \text{(Equ. 2.3)}$$

The y axis intercept *b* is equal to P concentrations in the non-fumigated aliquot. In accordance with Oberson and Joner (2005) and Bünemann et al. (2008) this study did not use a transformation factor for the calculated Phex concentrations and used Pmic synonymously for Phex. For the measurements hexanol was used instead of chloroform, because it is just as effective as chloroform but having a less hazardous nature (McLaughlin et al. 1986). Noteworthy, Bergkemper et al. (2016) reported lower Pmic concentrations of hexanol fumigation as compared to chloroform fumigation calling for cautious comparisons among different fumigation agents. Studies by Bünemann et al. (2004) showed that neither the P concentration nor the specific activity of ^{33}P in resin-P were affected by the presence of hexanol during extraction and that the addition of the plant residue (e. g. roots) did not represent a source of error for the determination of Pmic by hexanol fumigation of soils.

Soil microbial biomass carbon (Cmic) and nitrogen (Nmic) were determined using the chloroform fumigation extraction method (Vance et al. 1987). Briefly, 10 g soil subsamples were fumigated with chloroform for 24 h and then extracted with 40 ml 0.5 M K_2SO_4 on a horizontal shaker for 30 min at 250 rpm and centrifuged for 30 min at 4400 g. A second sample remained non-fumigated, but was treated identically. Carbon (C) and N in supernatants were measured with a DOC/TN-analyser (Multi N/C 2100S, Analytik Jena, Jena, Germany). Carbon and N content of the unfumigated samples were deducted from the fumigated samples to calculate the microbial share of C and N.

For P fractions in soil, data published by Alt et al. (2011) were used. Because of inaccessibility of some sites (e. g. because of extraordinarily high soil moisture), for P fraction measurements some of the 300 sites in total could not be sampled resulting in a reduced data set (forest *n* = 131, grassland *n* = 110; Alt et al. (2011)). Different P fractions of 0.5 g air-dried soil samples were measured after a method described by Hedley et al. (1982) modified by Kuo (1996). The first fraction extracted with anion exchange resins (resin-P) was not analyzed separately but is included in the $NaHCO_3$-extractable fraction. The further sequential extraction scheme had four steps ($NaHCO_3$-P, NaOH-P, HCl-P, H_2SO_4-P). Firstly, 20 ml 0.5 M $NaHCO_3$ (pH = 8.5) was added to the sample and the suspension was

shaken for 30 min, decanted, and filtrated. Secondly, 30 ml 0.1 M NaOH was added to the remaining soil followed by shaking for 16 h, decantation and filtration. Thirdly, the remaining soil was mixed with 0.1 M HCl, heated in a water bath (80 °C, 30 min) and cooled down (1 h) followed again by decantation and filtration. Fourthly, the residual soil mass was muffled overnight in a porcelain crucible in a muffle furnace at 550 °C to destroy all organic material. Thereafter, 20 ml 0.5 M H_2SO_4 was added and the suspension was shaken for 16 h, decanted, and filtrated. The decanted solution of each step was analysed for P concentrations. All Pi contents were analysed using the ammonium molybdate-ascorbic acid blue method described by Murphy and Riley (1962) and measured with a continuous flow analyser (CFA, AA3, XY2, Seal-Analytic, Norderstedt, Germany) at λ = 660 nm. Total dissolved P in $NaHCO_3$-extract and NaOH-extract was measured with an Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES, PerkinElmer Optima 5300 DV, S10 auto sampler, λ = 213.617 nm). For the labile and moderately labile fractions ($NaHCO_3$-P, NaOH-P), Po was calculated by subtracting Pi from total dissolved P concentrations in the extracts. In addition to the individual fractions, Pi in the labile and moderately labile fraction was calculated as the sum of $NaHCO_3$-Pi and NaOH-Pi (Σ $NaHCO_3$-Pi + NaOH-Pi) and Po in the labile and moderately labile fraction as the sum of $NaHCO_3$-Po and NaOH-Po (Σ $NaHCO_3$-Po + NaOH-Po). Total P concentrations represent the sum of all P fractions. The soil pH was measured using 0.01 M calcium chloride and a soil : solution ratio of 1:2.5. In order to analyse total C and total N contents of soils, subsamples were ground in a ball mill to homogenize the material. Total C and N concentrations were then determined by dry combustion in an elemental analyzer (VarioMax CN analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). Similarly, inorganic C was measured with the same analyzer after ignition of Corg at a temperature of 450 °C for 16 h (Solly et al. 2014). Subsequently, Corg was calculated as the difference between total and inorganic C.

Potential alkaline (APase) and acid (AcidPase) phosphomonoesterase activity in soil was determined after Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) using *p*-nitrophenol at pH 11 (APase) and pH 6 (AcidPase) and a photometer (Specord®200 Plus, AnalytikJena, Jena, Germany) at λ = 400 nm. For the determination of resin-P, 120 g of soil was filled into a two-liter PE bottle, six resin stripes (6 cm × 6 cm, anion exchange membranes, product code: 551642S VWR, Bruchsal, Germany) conditioned with 0.5 M $NaHCO_3$ along with 1.5 L deionized water were added, and the suspension was shaken for 24 h (Weiner et al. 2011). 0.2 M HNO_3 was used to desorb Pi from the resin. A small aliquot of extraction solution was used for the measurement of Pi concentrations in extracts by means of the molybdate blue method (Murphy and Riley 1962).

In the remaining aliquot of extraction solutions, Pi was separated from interfering compounds according to Tamburini et al. (2010), yielding silver phosphate (Ag_3PO_4). The precipitation was done in 4 steps: (1) ammonium phospho-molybdate mineral precipitation and dissolution, (2) magnesium ammonium phosphate mineral precipitation and dissolution, (3) cation removal, (4) precipitation as

Ag_3PO_4. Precipitated Ag_3PO_4 was analyzed for $\delta^{18}O$ values ($\delta^{18}O_{Pi}$) using a high-temperature elemental analyzer (Thermo-Quest TC-EA, Tübingen, Germany) coupled with a mass spectrometer (Thermo-Quest Delta+XL, Tübingen, Germany). Samples were calibrated to $\delta^{18}O$ values of TU-1- ($\delta^{18}O$ = 21.11 ‰), TU-2- ($\delta^{18}O$ = 5.35 ‰) and YR-1a- ($\delta^{18}O$ = -5.77 ‰) internal laboratory standards, each related to the VSMOW (Vienna Standard Mean Ocean Water) international standard (International Atomic Energy Agency 1995). The reproducibility for $\delta^{18}O$ measurements was ± 0.3 ‰. The yield of the standards was on average 97.8 % with a standard deviation of 1.5 %. The measured samples had a yield of 88.5 ± 1.1 %. In addition, $\delta^{18}O_{SW}$ extracted by cryogenic vacuum extraction (Schmidt et al. 2012, Orlowski et al. 2013) were measured with cavity ring down spectroscopy (DLT-100-Liquid Water Isotope Analyzer, Los Gatos Research Inc., Mountain View, CA, USA) at Giessen.

2.6 Vegetation records

Plant diversity was measured as the number of vascular plant species in a sampling plot. In 2009, vascular plants were sampled in all plots. In grasslands, this survey was conducted once between mid-May and end-of-June in an area of 4 m × 4 m (Socher et al. 2012), when the vegetation was fully developed and prior to the meadows being mown. In forests, vegetation changes strongly during the seasons with high abundances of early flowering geophytes in spring and high abundances of later flowering species in summer. In order to account for these differences and for sampling the total plot diversity, the vegetation was recorded in all plots in two surveys in spring (April) and late summer (June – July) of the same year, in an area of 20 m × 20 m. The spring and summer records were combined for assesssing the total plant diversity per plot (Boch et al. 2013).

2.7 Calculation and statistics

The significance level was set to $P < 0.05$ if not specified otherwise. All statistical analyses were conducted using IBM SPSS Statistics 23, structural equation models were calculated with IBM SPSS AMOS 23 for Windows, respectively. Figures were drawn with R Version 3.3.2 using “ggplot2” (Wickham 2009), “stats” (R Core Team 2016) and “stringi” (Gagolewski and Tartanus 2016). Some data were obtained from the Biodiversity Exploratories Information System (BExIS, www.bexis.uni-jena.de) after consultation with the data-owner. All utilised BExIS datasets in this thesis are listed in **Table 2.3**.

Table 2.3: Data obtained from the Biodiversity Exploratories Information System (BExIS).

data information	BExIS ID	reference
soil phosphorus fractions	5241	Alt and Oelmann (2010)
vegetation records (forest site)	6240	Boch et al. (2010)
vegetation records (grassland site)	6340	Prati et al. (2016)
fertilization and land use (grassland site)	13726	Gockel and Weisser (2017)
mineral soil C and N concentrations (2011)	14446	Schöning and Trumbore (2014)
mineral soil texture	14686	Schöning et al. (2011)
pH, microbiological properties (2011)	14766	Berner et al. (2014)
microbial phosphorus (2011)	15766	Sorkau and Oelmann (2015a)
mineral soil C and N concentrations (2014)	18787	Schöning et al. (2015)
climate data	19007	Wöllauer et al. (2015)
pH (2014)	19067	Schöning and Trumbore (2015)
land-use information (grassland site)	19746	Lorenzen and Weisser (2016)
enzyme activity (2011)	20246	Kandeler et al. (2017a)
enzyme activity (2014)	20247	Kandeler et al. (2017c)
microbiological properties (2014)	20251	Kandeler et al. (2017b)
microbial phosphorus (2014)	20286	Sorkau and Oelmann (2016)
land use (forest site)	20826	Nieschulze et al. (2017)

Microbial phosphorus

In order to test for significant differences in mean Pmic concentrations among regions and land-use types, an analysis of variance by means of *Tukey* Post Hoc tests (Janssen and Laatz 2013, Tukey 1962) was used. Correlations among variables were studied using *Pearson's* rank correlations (Pearson 1896, 1920). For disentangling complex interactions among variables controlling Pmic in grassland and forest soils and to overcome limitations associated with bivariate correlations, structural equation models (SEM) (Grace et al. 2012) were applied. Different models were structured in order to check for the direct and indirect effects on Pmic and disentangling driving factor Corg (Model 1: Corg influencing Pmic and plant diversity) and plant diversity (Model 2: plant diversity influencing Pmic and Corg), respectively. As an evaluation criterion for model quality the probability level and root mean square error of approximation (RMSEA) was observed (Chai and Draxler 2014). Reducing the number of abiotic variables for the SEM, a principal component analysis (PCA) with varimax rotation was calculated. The prerequisites for a PCA were checked by Bartlett's Test of Sphericity (Bartlett *P*) and the Kaiser-Meyer-Olkin index (KMO) (Abdi and Williams 2010). The threshold for meaningful components was set to Eigenvalues > 1. Thereafter, those variables were identified, that were most important within one component and thus, can be considered representative of other variables in the same component. As selection criterion, the highest loading of the variables in the components was used.

Some Pmic concentration data points were removed via Grubbs and Beck (1972) outlier test from the 2011 data set (number of outliers: ALB: grassland = 2, forest = 2; HAI: grassland = 4, forest = 3; SCH: grassland = 4, forest = 12).

Quantification of enzyme-mediated hydrolysis

All isotopic values are given in delta-notation in per mil (‰) versus VSMOW. The deviation of $\delta^{18}O_{SW}$ and $\delta^{18}O_{Pi}$ values at a given point in time during incubation from initial (t = 0) $\delta^{18}O_{SW}$ and $\delta^{18}O_{Pi}$ values was tested using a matched pairs t-test (Janssen and Laatz 2013). Differences in $\delta^{18}O_{SW/ti}$ and $\delta^{18}O_{Pi/ti}$ values among treatments (= addition of isotopically labeled water) and potential changes during the incubation were tested using a repeated measures ANOVA (analysis of variance) (von Ende 2001). The repeated measures ANOVA including periods of time as within-subject factor and treatment as between-subject factor and an interaction term of period of time and treatment. For particular points in time of incubation, a univariate ANOVA was conducted. In all cases, the prerequisite for the analysis of variance were met (homoscedasticity as proven by a *Levene* test (Janssen and Laatz 2013)).

Manipulating $\delta^{18}O_{SW}$, while keeping all other factors constant, results in a linear relationship between $\delta^{18}O_{SW/ti}$ and $\delta^{18}O_{Pi/ti}$ given as **(Equ. 2.4)**:

$$\delta^{18}O_{Pi/ti} = a_{ti} \cdot \delta^{18}O_{SW/ti} + b_{ti} \qquad \text{(Equ. 2.4)}$$

where *a* is the slope and *b* is the y intercept of the regression. It is generally assumed that the fractionation factor of enzymes involved in cleavage of Pi does not depend on the $\delta^{18}O$ values of ambient water and thus, is identical for the different isotopically labeled waters (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015). Therefore, the slope *a* represents the total biological incorporation of water-O in Pi, i. e. the fraction of O isotopes in PO_4 which was exchanged. As a measure of robustness, *a* was calculated from regressions based on data of the different treatments, i. e. either two-point (LM, LH, MH) or three-point (LMH) regressions.

The expected values for $\delta^{18}O_{Pi}$ values from thermodynamic equilibrium fractionation ($\delta^{18}O_{Pi/Equ}$) were calculated according to Chang and Blake (2015) **(Equ. 2.5)**.

$$\delta^{18}O_{Pi/Equ} = (\delta^{18}O_{SW} + 1000) \cdot e^{\left(\frac{14.43 \cdot 1000}{T - 26.54}\right)} - 1000 \qquad \text{(Equ. 2.5)}$$

where *T* denotes the temperature in kelvin.

Kinetic fractionation occurs in case of extracellular enzyme-mediated Pi release in soil. Monoesterase activity is generally assumed to dominate over other phosphatase activities in soil (Nannipieri et al. 2011, Margesin and Schinner 1994). Monoesterase activity results in the inheritance of three out of four O atoms from the Po substrate in released Pi while one O atom is incorporated from ambient water associated with kinetic fractionation (Blake et al. 2005). Therefore, $\delta^{18}O$ values to be expected from kinetic fractionation ($\delta^{18}O_{Pi/kin}$) can be calculated based on **(Equ. 2.6)**:

$$\delta^{18}O_{Pi/kin} = 0.75 \cdot \delta^{18}O_{Po} + 0.25 \cdot \left(\delta^{18}O_{SW/ti} + \varepsilon_{Pi/SW}\right) \quad \text{(Equ. 2.6)}$$

with $\delta^{18}O$ values of soil water ($\delta^{18}O_{SW}$) at a given point in time (ti) during incubation. For $\delta^{18}O$ values of Po and the fractionation factor $\varepsilon_{Pi/W}$, the mean values ($\delta^{18}O_{Po}$ = 15 ‰, $\varepsilon_{Pi/W}$ = -15 ‰) were used published by Liang and Blake (2006b) and von Sperber et al. (2014), (2015). Additonally, a sensitivity analysis was conducted including the full range of $\delta^{18}O_{Po}$ and $\varepsilon_{Pi/W}$ values and of diesterase activity in the result and discussion section (**section 3.3**) of this thesis.

$\delta^{18}O_{Pi/ti}$ and $\delta^{18}O_{Pi/t0}$ can be measured directly. The total incorporation factor *a* can be assessed based on the use of two or more waters differing in $\delta^{18}O_W$ values (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015). $\delta^{18}O_{Pi/Equ}$ can be calculated after Chang and Blake (2015). In order to calculate $\delta^{18}O_{Pi/kin}$, the isotopic fractionation factors and the isotopic signature of the Po source being hydrolyzed must be known, which has to be retrieved from the literature (Liang and Blake 2006b, 2009, von Sperber et al. 2014). Accordingly, the reliability of this approach requires additional attention. Finally, the kinetic O incorporation factor *c* **(Equ. 1.4)** remains the only unknown and the equation can be rearranged to solve for *c*.

3 Results and discussion

3.1 The role of soil chemical properties, land use and plant diversity for microbial phosphorus in forest and grassland soils

Management intensity modifies soil properties, e. g. Corg concentrations and soil pH with potential feedbacks on plant diversity. These changes might influence Pmic concentrations in soil representing an important component of the P cycle. The objectives were to elucidate whether abiotic and biotic variables controlling Pmic concentrations in soil are the same for forests and grasslands, and to assess the effect of region and management on Pmic concentrations in forest and grassland soils as mediated by the controlling variables. In three regions of Germany, ALB, HAI and SCH, forest and grassland plots (each *n* = 150, year 2011) differing in plant diversity and land-use intensity were studied. In contrast to controls of Cmic, Pmic was strongly influenced by soil pH, which in turn affected P availability and thus microbial P uptake in forest and grassland soils. Furthermore, Pmic concentrations in forest and grassland soils increased with increasing plant diversity. Using SEMs, it could be shown that soil Corg is the profound driver of plant diversity effects on Pmic in grasslands. For both forest and grassland, regional differences in Pmic attributable to differing environmental conditions (pH, soil moisture) were found. Forest management and tree species showed no effect on Pmic due to a lack of effects on controlling variables (e. g. Corg). Also, no management effects in grassland soils were found which might be caused by either compensation of differently directed effects across sites or by legacy effects of former fertilization constraining the relevance of actual practices. So, this study concluded that variables controlling Pmic or Cmic in soil differ in part and that regional differences in controlling variables are more important for Pmic in soil than those induced by management. Results are shown as mean [± standard deviation] if not specified otherwise.

3.1.1 Forests

Across all forest soils, Pmic concentrations increased with increasing concentrations of NaOH-Po, Σ $NaHCO_3$-Po + NaOH-Po, HCl-P, H_2SO_4-P, total P, total N, Corg, Cmic, Nmic concentrations, as well as pH and plant diversity, and decreased with increasing $NaHCO_3$-P_i, $NaHCO_3$-P_o concentrations, C : N ratio and Cmic : Nmic ratio (**Table 3.1**). Microbial P concentrations correlated significantly positively with soil moisture and clay contents (**Table 3.1**).

Table 3.1: Relationship of different biotic and abiotic variables to Pmic in forest and grassland soils (*r* = *Pearsons*'s rank correlation, *n* = sample size; ns = not significant, * $P < 0.05$ ** $P < 0.01$, *** $P < 0.001$).

land-use type		forest soils		grassland soils	
variable		*r*	*n*	*r*	*n*
abiotic	clay	0.83***	133	0.33***	140
	medium silt	0.33***	133	0.29**	140
	medium sand	-0.58***	133	-0.38***	140
	soil moisture (10 cm below surface)	0.80***	133	0.41***	140
	$NaHCO_3$-Pi	-0.37***	117	-0.38***	108
	$NaHCO_3$-Po	-0.51***	117	ns	
	NaOH-Pi	ns		ns	
	NaOH-Po	0.57***	117	0.38***	108
	HCl-P	0.71***	117	0.22*	108
	H_2SO_4-P	0.71***	117	0.22*	108
	Σ $NaHCO_3$-Pi + NaOH-Pi	ns		ns	
	Σ $NaHCO_3$-Po + NaOH-Po	0.48***	117	0.38**	108
	total P	0.72***	117	0.28**	108
	pH	0.72***	118	0.30***	131
	Corg	0.65***	133	0.44***	140
	total N	0.86***	133	0.48***	140
	C : N	-0.55***	133	ns	
biotic	Nmic	0.71***	132	0.57***	140
	Cmic	0.86***	132	0.63***	140
	Cmic : Nmic	-0.26**	132	ns	
	plant diversity	0.45***	132	ns	

At the same time, soil moisture was higher in fine-textured soils (**Supplementary material: Table S 1**). The ratios of Cmic : Pmic ranged from 5 to 213 with an average of 22 (median = 16). With increasing plant diversity, $NaHCO_3$-Pi concentrations and Cmic : Pmic ratios decreased (r = -0.19 and -0.27, respectively; $P < 0.05$). The three regions differed in their overall Pmic concentrations in the order ALB > HAI > SCH (**Figure 3.1**a)

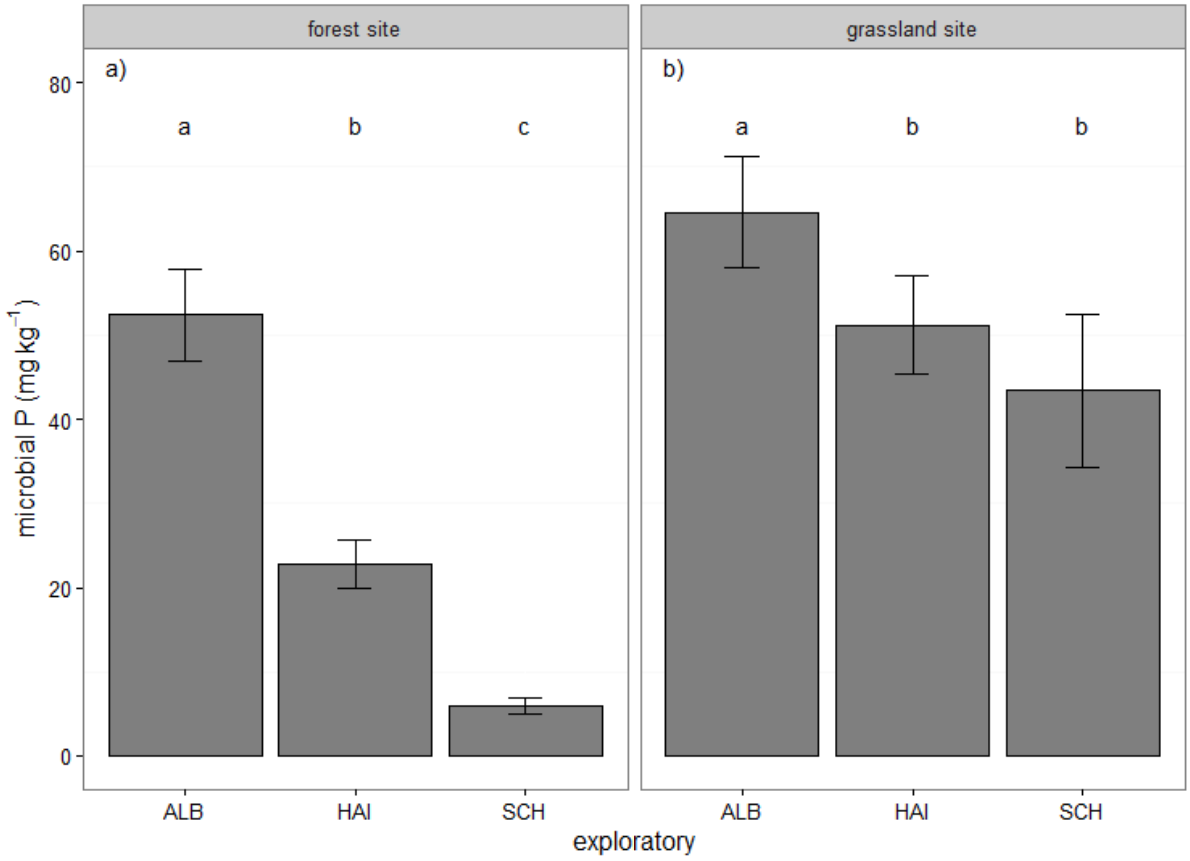

Figure 3.1: Mean concentrations of Pmic in a) forest and b) grassland soils from three exploratory regions in Germany (ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin; error bars represent standard errors; different letters represent significant differences [$P < 0.05$]).

Separated according to region, neither forest management influenced Pmic concentrations significantly nor did tree species ($P > 0.05$; **Table 3.2**). For forests, the prerequisites for a PCA to reduce the number of variables in a SEM were fulfilled (KMO = 0.40, Bartlett $P < 0.001$). The PCA resulted in three components explaining 78.9 % of the variance in the data. Based on the highest loading in each component, the following variables were selected: total N concentrations (Component 1; loading 0.91), $NaHCO_3$-P_i concentrations (Component 2; loading 0.90), NaOH-P_i (Component 3; loading 0.94). If data sets of grasslands and forests were merged, the PCA identified two further influential variables, i. e. Corg concentrations (loading 0.91) and soil pH (loading 0.85). Because of similar loadings (and a close bivariate correlation between total N and Corg concentrations; $r = 0.98$, $P < 0.001$) and the desired consistency for grassland and forest SEMs, total N concentrations were replaced by Corg concentrations in the final model. Two SEMs were used treating plant diversity either as a response variable (depending among others on Corg concentrations, Model 1; **Supplementary material: Figure S 1**) or as a variable controlling Corg concentrations (**Supplementary material: Figure S 2**). For forest soils, SEMs could not be adequately fitted to Pmic concentrations as probability

levels < 0.02 and RMSEA > 0.3 indicated major deviations between data and models if regions were analysed separately (**Supplementary material**: **Table S 2**, **Figure S 1**, **Figure S 2**). The one exception was the SCH characterized by a better fit between data and model, which however did not include paths significantly explaining Pmic concentrations. If all regions were analysed together (all regions), the deviation between data and model was smaller (**Supplementary material**: **Table S 2**). In both models, paths including plant diversity were significant.

Table 3.2: Mean [± standard deviation] of Pmic concentrations differentiated to land use and region (in mg kg^{-1}; na = not available, different letters present significant differences within the exploratory [P <0.05]).

	land-use type	region					
		Schwäbische Alb		Hainich-Dün		Schorfheide-Chorin	
forest soils	tree species						
	beech	53.6[±18.6]	a	22.2[±9.7]	a	4.7[±2.7]	b
	spruce	48.7[±19.7]	a	29.9[±8.6]	a	na	
	oak	na		na		4.8[±1.4]	ab
	pine	na		na		7.2[±2.9]	a
	management						
	age class forest	51.2[±18.8]	a	24.6[±10.7]	a	5.8[±2.9]	a
	unmanaged forest	66.0[±13.3]	a	24.5[±8.9]	ab	6.7[±3.7]	a
	selection cutting	na		15.6[±2.7]	b	na	
grassland soils	fertilization						
	yes	63.4[±24.1]	a	49.7[±24.2]	a	46.0[±26.5]	a
	no	66.3[±20.7]	a	52.7[±14.7]	a	42.7[±32.0]	a
	management						
	meadow	64.2[±23.7]	a	44.2[±25.1]	a	53.1[±29.3]	a
	mown pasture	64.6[±25.7]	a	52.9[±22.0]	a	52.2[±35.5]	a
	pasture	65.1[±21.0]	a	52.0[±15.6]	a	31.3[±26.3]	a
	number of cuts per year						
	0	66.1[±21.0]	a	52.6[±14.8]	a	31.3[±26.3]	a
	1	70.5[±22.5]	a	54.1[±25.4]	a	46.7[±35.2]	a
	2	64.8[±25.6]	a	39.6[±8.5]	a	60.0[±25.1]	a
	3	51.7[±18.9]	a	26.3[±5.1]	a	58.7[±30.2]	a

3.1.2 Grasslands

Overall, Pmic increased with increasing pH, concentrations of NaOH-Po, total P, Σ $NaHCO_3$-Po + NaOH-Po, Nmic, total N, Cmic, Corg, and plant diversity and decreased with increasing $NaHCO_3$-Pi concentrations (**Table 3.1**). Pmic concentrations were related to soil moisture and clay contents (**Table 3.1**) while soil moisture was higher in fine-textured soils (**Supplementary material: Table S 1**). In grassland soils, a mean Cmic : Pmic ratio of 14 (median = 12) with a range of 2 to 118 was measured. With increasing plant diversity, $NaHCO_3$-Pi and Cmic : Pmic ratio decreased (r = -0.62 and -0.24, respectively; P < 0.01).

Mean concentrations of Pmic in grassland soils of the three different regions followed the same order as in forest soils: ALB > HAI ≥ SCH (**Figure 3.1**b). Microbial P concentrations among management types showed high variability (range in meadows: 11.6 to 105.8 mg kg^{-1}, mown pastures: 19.1 to 114.1 mg kg^{-1}, pastures: 6.1 to 103.0 mg kg^{-1}) but were not significantly different. Phosphorus fertilization rates were low (see **Section 2.2**) and there were also no significant differences in Pmic concentrations between fertilized (55.4 [±3.2] mg kg^{-1}) and non-fertilized soils (51.6 [±3.0] mg kg^{-1}) in general (specified to exploratory: **Table 3.2**). In addition, no influence of cutting frequency on Pmic concentrations was found (0 cut/year: 49.3 [±3.2] mg kg^{-1}, 1 cut/year: 55.2 [±4.6] mg kg^{-1}, 2 cuts/year: 60.5 [±4.6] mg kg^{-1}, 3 cuts/year: 47.6 [±7.1] mg kg^{-1}) (specified to exploratory: **Table 3.2**).

For grasslands, the prerequisites for a PCA to reduce the number of variables in a SEM were fulfilled (KMO = 0.26, Bartlett $P < 0.001$). The PCA resulted in three components explaining 69.4 % of the variance in the data. Based on the highest loading in each component, the following variables were selected: NaOH-Pi (Component 1; loading 0.81), HCl-P (Component 2; loading 0.90), $NaHCO_3$-Pi concentrations (Component 3; loading -0.81). In addition, HCl-P concentrations were replaced by the variable with the second-highest loading in Component 2, i. e. total P concentrations (loading 0.84). Thus, the SEM included the same variables as described above for forests. Most of the SEMs showed an adequate fit between data and model (**Supplementary material: Table S 2**) with a mean explained variance of Pmic of 34 % (median = 26 %) for Model 1 (**Figure 3.2**) and 25 % (median = 20 %) for Model 2 (**Figure 3.3**). For the models across all regions (Grass 2011: **Figure 3.2**, **Figure 3.3**), a significant influence of plant diversity on Pmic concentrations in soils was found. If the regions were analysed individually, however, this direct effect of plant diversity was no longer significant (ALB, HAI, SCH: **Figure 3.2**, **Figure 3.3**). For Model 1 in which plant diversity was considered as dependent on Corg concentrations and in case of Grass 2011, AEG 2011 and HEG 2011 (**Figure 3.2**), Corg concentrations in soil were related to both, plant diversity and Pmic concentrations in soil. However, plant diversity was related indirectly to Pmic concentrations as i) there was no significant direct path if regions were analysed individually, and ii) plant diversity significantly explained a proportion of variance in Corg concentrations which itself was an important explanatory variable of Pmic concentrations (**Figure 3.2**, **Figure 3.3**).

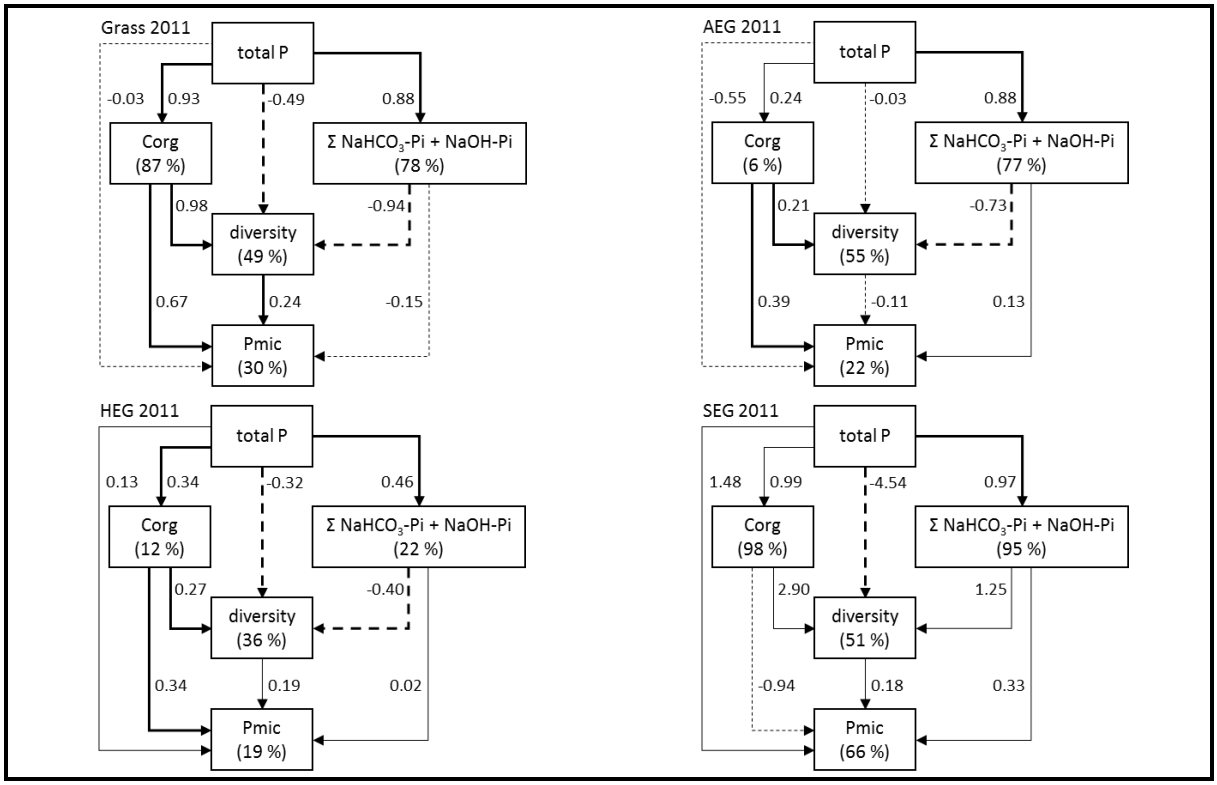

Figure 3.2: Structural equation model 1 of the relationships between different abiotic controlling factors, plant biodiversity (diversity) and microbial phosphorus (Pmic) in grassland sites for Grass (grassland site all three regions together, RMSEA = 0.081), AEG (grassland site Schwäbische Alb, RMSEA < 0.001), HEG (grassland site Hainich-Dün, RMSEA < 0.001) and SEG (grassland site Schorfheide-Chorin, RMSEA < 0.001). In this model, plant diversity is considered as response variable, i. e. depending on organic carbon (Corg) concentrations. The percentage of explained variance of the variable is given in brackets. Numbers next to arrows represent standardized path coefficients. Path lines: bold lines = $P < 0.05$; thin lines = $P \geq 0.05$; solid line = positive path coefficient; dashed line = negative path coefficient.

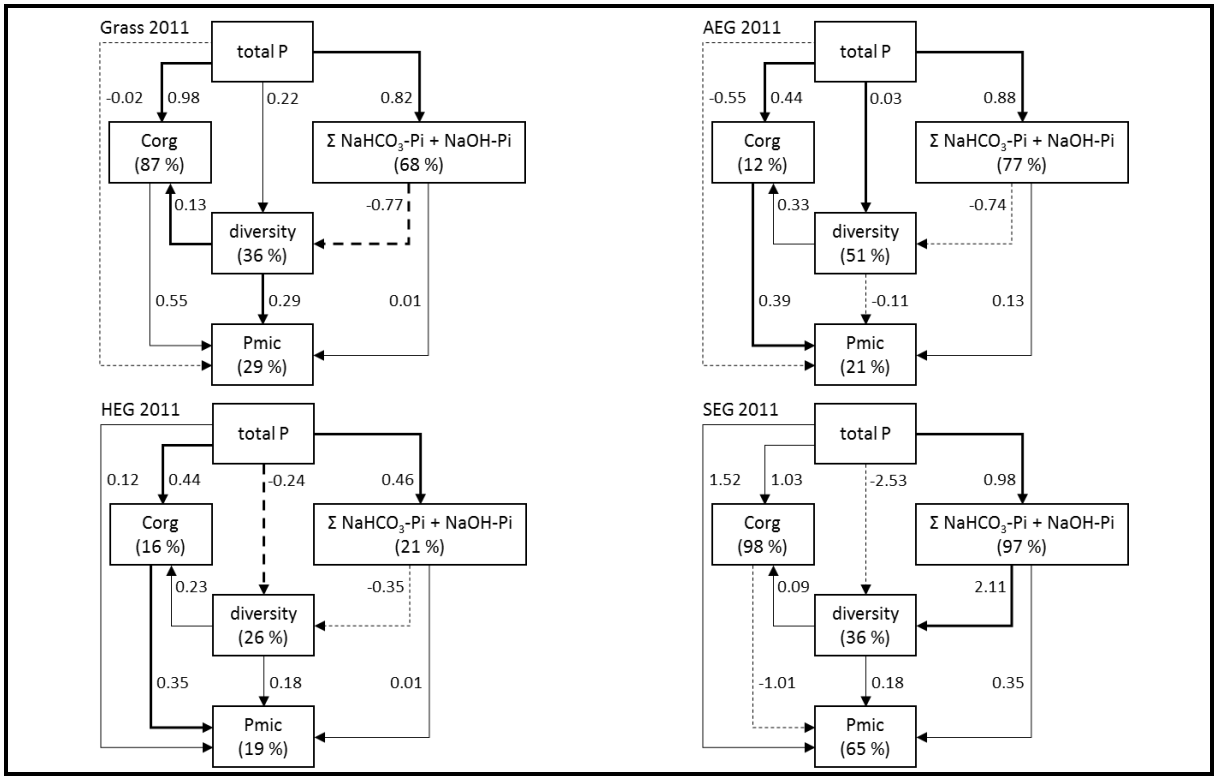

Figure 3.3: Structural equation model 2 of the relationships between different abiotic controlling factors, plant biodiversity (diversity) and microbial phosphorus (Pmic) in grassland sites with plant diversity as driving factor for Grass (grassland site all three regions together, RMSEA = 0.271), AEG (grassland site Schwäbische Alb, RMSEA < 0.001), HEG (grassland site Hainich-Dün, RMSEA = 0.180) and SCH (grassland site Schorfheide-Chorin, RMSEA < 0.001). In this model, plant diversity is considered as independent variable, i. e. controlling both organic carbon (Corg) and Pmic concentrations. The percentage of explained variance of the variable is given in brackets. Numbers next to arrows represent standardized path coefficients. Path lines: bold lines = $P < 0.05$; thin lines = $P \geq 0.05$; solid line = positive path coefficient; dashed line = negative path coefficient.

3.1.3 Variables controlling Pmic concentrations in soil

Variables controlling Pmic concentrations in soil were suspected to be partly identical to those driving Cmic concentrations in soil. Indeed, for both forests and grasslands, soil moisture, Corg concentrations, pH, and total P controlled Pmic concentrations partly corroborating findings on factors driving Cmic concentrations in soil (Wardle 1992, 1998, Joergensen et al. 1995). Reviews on controls of microbial biomass confirm the positive effect of soil moisture (up to a threshold) on microbial biomass (Wardle 1992) likely because microbes need water for their metabolism. However, this relationship not necessarily is universal because site-specific properties such as e. g. microbivory might play a role as well (Wardle 1992). The results highlight the importance of soil moisture for Pmic concentrations. Increasing soil moisture results in an increasing diffusivity of P enhancing the uptake by microbes and plants (Lambers et al. 2006). In this study, soil moisture was related inversely to soil texture (**Supplementary material: Table S 1**). Therefore, variability in soil moisture among sites was caused by soil texture and in addition very likely by preceding weather conditions. Because of the covariation

between soil moisture and soil texture, a relationship between soil texture and Pmic concentrations as well (**Table 3.1**) was found. Because of the experimental design (highest moisture linked to finest texture and lowest soil moisture linked to coarsest texture), there was was no ability to tease apart the effects of the two variables. In line with the effect of Corg, phospholipid fatty acids (forest site: Herold et al. (2014b)) and enzyme activities (grassland site: Boeddinghaus et al. (2015)) were mainly related to Corg concentrations. Soil pH effects were as strong as those of Corg concentrations (**Table 3.1**). Microorganisms take up P in the form of $H_2PO_4^-$ ions and the ion concentration in solution is controlled among others by the pH-dependent solubility product of P-containing minerals (Brookins 1988). Therefore, soil pH acts as a control of P availability in soil in turn influencing the possibility of P uptake by microorganisms. In addition, labile P_i concentrations were negatively related to Pmic concentrations in soil (**Table 3.1**). This indicates P immobilization in microbial biomass, suggesting Pmic to act as a sink rather than a source of plant-available P in this study. Interestingly, relationships between P fractions and Pmic concentrations in soil were closer in forests as compared to grasslands (**Table 3.1**). In conjunction with higher Pmic concentrations (**Figure 3.1**), this might be indicative of higher microbial turnover and thus, higher contribution of microbial processes to P cycling in grasslands as compared to forests. A strong microbial contribution to P cycling was suggested for extensively used grasslands by Stutter et al. (2015) as well. Another difference between forest and grassland soils was related to N. In forests and grasslands, total N concentrations in soil explained 86 % and 48 % of the variation in Pmic concentrations, respectively (**Table 3.1**). Though one must keep the collinearity of Corg and total N concentrations in soil in mind, the greater relevance of N for microbial biomass (Cmic) in forests was already suggested by Wardle (1992), (1998). Based on the results the prominent role of N in forest soils is transferable to Pmic concentrations. Soil C : N ratios correlated significantly with Pmic concentrations in forest soils which was not the case for grasslands (**Table 3.1**) with a smaller range in soil C : N ratios as compared to forest soils (range in forests and grasslands, 11.1 to 26.3 and 9.0 to 14.6, respectively). In conclusion, the hypothesis namely that Corg concentrations and soil moisture influence Pmic concentrations in forest and grassland soils could be accepted. In addition, soil pH was a strong control of Pmic concentrations in both systems. Finally, total N concentrations, soil C : N ratios, and P fractions (except for NaOH-Pi) were particularly decisive for Pmic concentrations in forest soils.

In forest soils, Pmic concentrations increased with an increasing plant diversity (**Table 3.1**) while the relationship for grassland was marginally non-significant (r =0.14, P = 0.09). The different SEMs (Model 1 and Model 2) revealed, however, that the relationship between plant diversity and Pmic resulted from simultaneous and positive relationships of Corg concentrations on both plant diversity and Pmic in grassland soils. However, this applied for mineral soil types in ALB and HAI only as high Corg concentrations in soil associated with organic soil types such as fens in SCH (Herold et al. 2014c)

revealed no link between plant diversity and Corg or Pmic concentrations in grassland soil. For the mineral soil types in the ALB and HAI, it was suggested that the effect of plant species diversity on Pmic concentrations is generated by an increase in resource availability, such as OM deposition by dead plant roots and microorganisms (Lange et al. 2015), rather than a direct causal effect of plant diversity on Pmic. Increased OM accumulation under diverse plant mixtures was proven based on a plant diversity experiment in grassland sites with random species selection for mixture compositions (Steinbeiss et al. 2008). Increased Corg concentrations in soil concomitantly increased soil microbial biomass/Pmic and activity in diverse mixtures (Eisenhauer et al. 2010, Hacker et al. 2015, Lange et al. 2015). Furthermore, a higher leaf area index associated with higher plant diversity resulted in higher soil moisture in the top soil layer (Lange et al. 2014) favoring higher Pmic concentrations in soil as was shown in this study. The results suggest that the positive relationship between OM accumulation and plant diversity might be transferable from artificial grassland mixtures to established grasslands thus, confirming the hypothesis that plant diversity has an effect on Pmic concentrations in addition to soil properties.

3.1.4 Regional differences and management effects

In general, Pmic concentrations were higher in grassland than in forest soils (**Figure 3.1**). This is in line with the results of Chen et al. (2003) and Yeates and Saggar (1998), who studied Pmic in mineral soils in forests and grasslands in New Zealand. One explanation might relate to the increased rhizosphere space that can be colonized by microbes. In line, Solly et al. (2014) found that grassland have higher fine root biomass, higher root turnover and higher rates of root decomposition compared to forest soils for this study site. Another explanation for low Pmic concentrations in forest plots may be the strong stratification of microbial colonization with soil depth, i. e. the well-developed organic litter layers on the soil surface contain higher Pmic concentrations than the mineral soil (Chen et al. 2003, Joergensen and Scheu 1999). Though Pmic was not measured in the organic layer, the fact that plant-available Pi concentrations were 6 to more than 20 times higher in the organic layer than in mineral soil (Zavišić et al. 2016) might serve as a corroboration of this stratification. Finally, differences in Corg concentrations between forests and grasslands could underly differences in Pmic concentrations (Corg – grasslands: 72.19 [±64.17] mg kg^{-1}, forests: 39.51 [±20.11] mg kg^{-1}). This was corroborated if analyzed per region as there were significant differences in both, Pmic and Corg concentrations between forests and grasslands in the HAI and SCH, but not in the ALB (in addition see Baumann et al. (2016)). Organic C and clay content in forest and grassland sites increased in the order SCH < HAI < ALB, a similar ranking was found for enzyme activities involved in the P cycle in forest soils (Herold et al. 2014a). Microbial P concentrations were highest in ALB for both forest and grassland soils (**Figure 3.2**). This trend

corresponds to the results of total phospholipid fatty acids in grassland soils by Herold et al. (2014b). Higher Pmic concentrations of the ALB might be explained by a combination of finer soil texture and higher annual precipitation, thus increasing soil moisture compared with the two other regions (ALB: 37.8 [±5.5] %, HAI: 32.2 [±4.8] %, SCH: 23.7 [±13.2] %). In addition to the reasoning provided in **section 3.1.3**, fine-textured soils are known to harbor more microbial biomass than coarse-textured soils (Naveed et al. 2016). Additionally, the ALB (followed by HAI) is characterized by i) higher Po and total P concentrations, and ii) higher soil pH in grassland and forest soils as compared to SCH (Alt et al. 2011). Therefore, the hypothesis on region-specific Pmic concentrations in soil which are caused by differences in environmental conditions among regions (Corg as substrate availability for microorganism metabolism, pH, soil moisture) could be confirmed.

Analysing the three regions separately, neither tree species nor management (managed vs. near-natural, **Table 3.2**) had significant effects on Pmic concentrations in forest soils. This might be caused by the fact that variables identified as controlling Pmic concentrations (see **Section 3.1.3**) did not show an effect either. For the study sites, no differences in Corg storage were observed in the density fractions among dominant tree species (Herold et al. 2014c). In addition, soil pH was not different between coniferous and broadleaved stands (data not shown; $P > 0.05$). This might be caused by management, e. g. in HAI spruce stands remained where the thickness of the Loess layer was greater as compared to stands that were reforested with beech. However, another factor identified as controlling Pmic concentrations in soil (see **Section 3.1.3**) differed between coniferous and broadleaved stands: the C : N ratio in mineral soil as an indicator of substrate quality and thus, microbial colonisation (data not shown: difference between spruce and beech 0.9 – 1.5 (ALB and HAI) and between pine and beech/oak 3.1 – 4.9 (SCH); $P < 0.05$). It could be inferred that the important role of pH for Pmic concentrations in soil overruled other less important drivers and prevented the occurrence of tree species effects. This reasoning is corroborated by the study of Aponte et al. (2013) where tree species effects (*Quercus canariensis* vs. *Quercus suber*) on Pmic concentrations in soil went along with differences in soil pH. Furthermore, mineral soil was analysed, but not the organic layer. Particularly in the organic layer, microbial properties (Cmic, Nmic, Cmic : Corg ratio, Nmic : total N ratio) were affected by the presence of coniferous tree species resulting from differences in litter C : N ratios and pH (Bauhus et al. 1998, Zhong and Makeschin 2006, Smolander and Kitunen 2011). In contrast, effects of tree species on mineral soil (e. g. pH, Cmic) were less pronounced or even absent (Bauhus et al. 1998, Zhong and Makeschin 2006). Zhong and Makeschin (2006) attributed the discrepancy between the organic layer and the mineral soil to the limited spatial extension of tree species effects in mineral soil which would have required a focus on the uppermost mineral soil (e. g. 0 – 3 cm). The same reasoning might apply to Pmic concentrations in the samples which – identical with the study of Zhong and Makeschin (2006) – were collected at a depth of 0 – 10 cm. In summary, the hypotheses on

tree-species/land-use intensity effects on Pmic concentrations in forest soils cannot unambiguously be rejected.

In accordance with results from forest soils, there was no effect of land-use types (pasture, mown pasture, meadow) or management measures (fertilization, cutting) on Pmic concentrations in grassland soils. For grassland sites land-use intensity affected the spatial structure of enzymes (Berner et al. 2011), but the influence on microbial biomass was not as large as expected and the individual site characteristic were more important (Boeddinghaus et al. 2015). In contrast to pronounced land-use effects such as grazing in subtropical grasslands (Wang et al. 2006, Devi et al. 2014) plants might regrow rapidly after animal browsing under temperate climate conditions. As a result, the proportion of bare area prone to erosion is restricted and the reduction of microbial biomass and accordingly, Pmic, by loss of soil due to erosion is minimized. Bristow and Jarvis (1991) found no difference between Cmic concentrations in grassland swards that were either grazed or cut (the latter associated with removal of the harvested material). They speculated that the lacking effect of reduced OM input into soil might be due to the fact that microbes in permanent grassland do not respond to small shifts in Corg availability in soil – an explanation that I consider reasonable given the generally high soil OM concentrations in grassland ecosystems. Similarly, there was no effect of fertilization on Pmic concentrations which contrasts with the study by Liebisch et al. (2014). In established ecosystems such as those within these study with long-lasting history of land use, current information on P fertilization rates might have limited relevance. Particularly for total P concentrations as one important driver of Pmic concentrations, legacy effects of former land use have to be taken into account (Smits et al. 2008, MacDonald et al. 2012). Therefore, not actual but aggregated fertilization rates over the last decades might better explain Pmic concentrations in grassland soils. Regrettably, such information is hardly available at all or if so associated with high uncertainties. Concluding, the hypothesis of a positive effect of land-use intensity and fertilization on Pmic concentrations in soil had to be rejected.

3.2 Microbial phosphorus of grassland sites – interannual variations

No differences for Pmic depending on land use were found (**Section 3.1**). For Pmic there are seasonal (climate) and spatial (chemical and physical) differences depending on environmental properties (Franklin and Mills 2003, Regan et al. 2014, Ritz et al. 2004, Zak et al. 2003). Considering the seasonal differences for Pmic (Regan et al. 2014) the investigations of Pmic was extended. The land use (meadow, mown pasture, pasture, N fertilization and cutting) at a given study area was not changed within three years (Lorenzen and Weisser 2016). Within the same season, but three years later I aimed (iii) to investigate if Pmic measurements are different interannually. Corg, which was disentangled as the driving factor, depends on soil type (with high Corg concentrations in Histosols (Eswaran et al. 1993)), vegetation and climate (Hartemink and McSweeney 2014). Therefore, (iv) Pmic investigations were extended on various soil types. Because of Corg is depending on soil type and Pmic is influenced by Corg I hypothesized (4) that there will be an effect of soil type on Pmic.

Soil samples were collected in May 2014 for the analysis of their Pmic content at the same sampling site as in May 2011 shifted for 10 cm (for details see **section 2.3**). Procedures for statistical analysis are described in detail in **section 2.7**. Additionally, in order to test for interanual differences a matched pairs t-test was used. All values are given as mean [± standard error] unless stated otherwise. Parallel to Pmic, microbiological properties (2011: Berner et al. (2014), 2014: Kandeler et al. (2017b)), Cmic and Nmic (2011: Schöning and Trumbore (2014), 2014: Schöning et al. (2015)), pH (2011: Berner et al. (2014), 2014: Schöning and Trumbore (2015)), enzyme activity (2011: Kandeler et al. (2017a), 2014: Kandeler et al. (2017c)) as well as land-use information (Lorenzen and Weisser 2016) was determined. Biodiversity and different P fractions were measured in 2009 and soil texture in 2011. The land use of the plots did not change between 2011 and 2014 (Lorenzen and Weisser 2016).

The soil sampling campaign was within the same season (spring) and month (02./05. – 11./15. May) of the year and there were no exceptional climatic events in the month prior to the sampling campaign (**Supplementary material: Table S 3**).

3.2.1 Microbial phosphorus 2011 vs. 2014

Pmic concentrations in soil

Compared to 2014 Pmic concentrations were significantly higher in 2011. Examining the Pmic concentration within these two years, a corresponding trend was found for both years depending on the exploratory (**Figure 3.4**): Pmic has the highest concentration in ALB and the lowest in SCH.

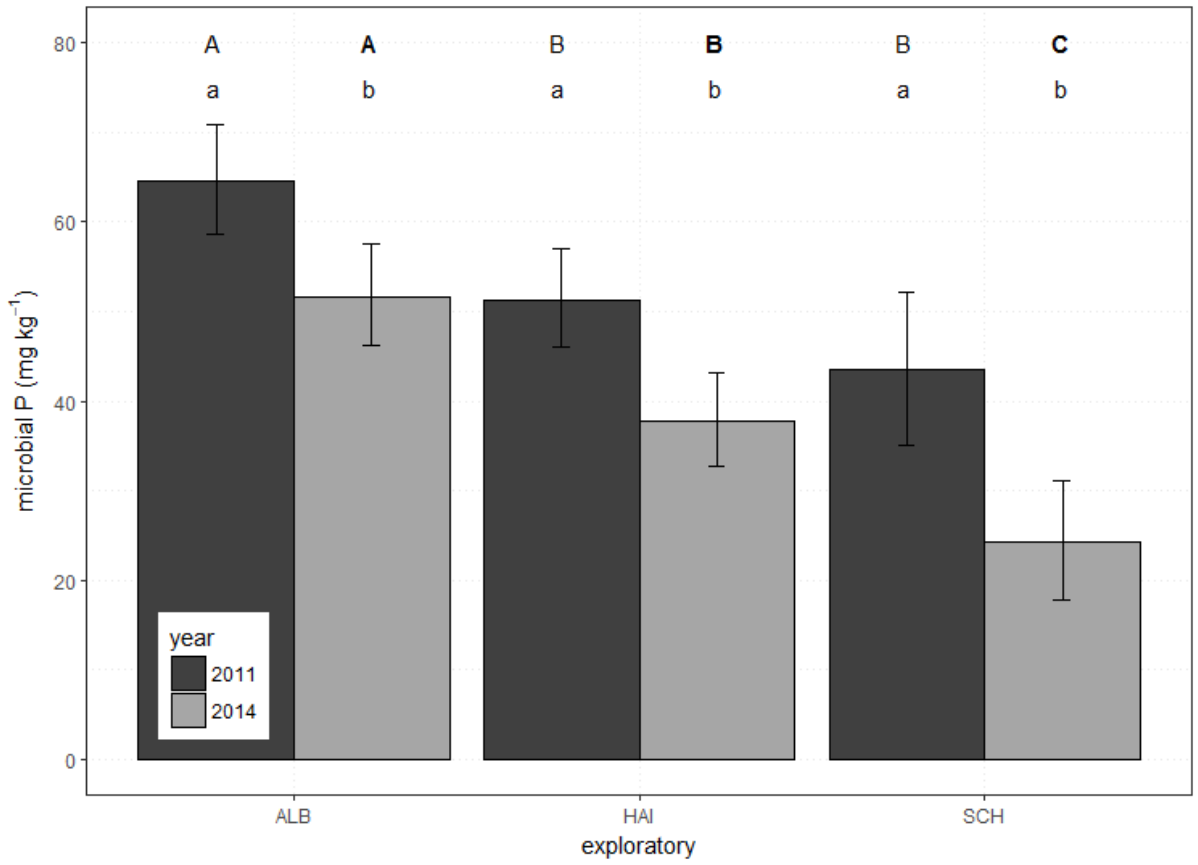

Figure 3.4: Mean concentrations of Pmic in grassland soils from three exploratory regions in Germany (ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin; error bars represent standard errors; different letters represent significant differences [$P < 0.05$], upper case letters regular: differences between exploratories in 2011, upper case letters bold: differences between exploratories in 2014, lower case letters: differences between years within exploratory).

Precipitation and soil moisture were significantly higher for 2014 than for 2011 within the exploratories (**Supplementary material: Table S 3**). Higher precipitation in 2014 April as well as May (**Supplementary material: Table S 3**) could be the explanation for lower Pmic concentrations in 2014. In case of higher precipitation, the soil pore volume will be stronger filled with groundwater. This led to formation of anaerobic zones, where microbial activity is less active in general (Karatas 2008, van Loodsrecht et al. 1987). Therefore, microorganisms in 2014 could be less productive, leading to less exoenzyme productivity (van Loodsrecht et al. 1987) and less Pmic. But this is in contast with the resin-P data. Resin-P for AEG (2011: 25.1 [±3.9] mg kg^{-1}, 2014: 35.9 [±3.8] mg kg^{-1}) and SEG (2011: 17.8 [±1.6] mg kg^{-1}, 2014: 26.7 [±2.3] mg kg^{-1}) are significant different but with increasing effect. Only in HEG the difference of resin-P is not significant ($P = 0.3$) and with decreasing effect, meaning 2014 (14.8 [±1.5] mg kg^{-1}) is slightly lower than 2011 (16.9 [±2.8] mg kg^{-1}). This phenomenon could be explained as follows: Higher precipitation results in higher soil moisture resulting in higher groundwater flow (Eltahir 1998, Hohenegger et al. 2009). Increasing groundwater flow induced higher microbial and nutrient losses with groundwater flow (Vadas et al. 2007), especially water-soluble

nutrients (e. g. resin-P (Blake et al. 2003)). Because the most active microorganisms are sessile (van Loodsrecht et al. 1990) groundwater induced nutrient losses will be compensate rapidly.

Variables controlling Pmic concentrations in soil

A detailed analysis of the correlation of Pmic to other biotic and abiotic factors yielded the same correlation trends for 2011 than 2014 (**Table 3.3**). For each region, the climate data of the sampling time are available (**Supplementary material: Table S 3**, except precipitation of 2011 in ALB). All climate data (May 2011 and May 2014) are significantly different (t-test, $P < 0.05$) interanually within the exploratories, except soil temperature in SCH ($P = 0.40$).

Table 3.3: Relationship of different biotic and abiotic variables to Pmic in grassland soils 2011 and 2014 (*r* = *Pearsons*'s rank correlation, *n* = sample size; ns = not significant, * $P < 0.05$ ** $P < 0.01$, *** $P < 0.001$).

land-use type		**grassland soils 2011**		**grassland soils 2014**	
variable		*r*	*n*	*r*	*n*
texture	clay	0.33***	140	0.48***	137
	fine silt	ns		0.31***	137
	medium silt	0.29**	140	ns	
	coarse silt	ns		ns	
	fine sand	-0.48***	140	-0.53***	137
	medium sand	-0.38***	140	-0.49***	137
	coarse sand	-0.22*	140	-0.37***	137
abiotic	soil moisture	0.41***	122	0.42***	124
	soil temperature	0.28*	78	-0.29**	110
	air temperature	ns		-0.32**	111
	$NaHCO_3$-Pi	-0.38***	108	-0.28**	103
	$NaHCO_3$-Po	ns		-0.25*	103
	NaOH-Pi	ns		0.31**	103
	NaOH-Po	0.38***	108	0.49***	103
	HCl-P	0.22*	108	0.40***	103
	H_2SO_4-P	0.22*	108	0.39***	103
	Σ $NaHCO_3$-Pi + NaOH-Pi	ns		0.24*	103
	Σ $NaHCO_3$-Po + NaOH-Po	0.38***	108	0.48***	103
	total P	0.28**	108	0.51***	103
	pH	0.30***	131	ns	
	Corg	0.44***	140	0.31***	137
	total N	0.48***	140	0.34***	137
	C : N	ns		ns	
biotic	Nmic	0.57***	140	0.50***	137
	Cmic	0.63***	140	0.61***	137
	Cmic : Nmic	ns		ns	
	phosphatase	0.17*	140	0.20*	137
	biodiversity	ns		ns	

So, 2014 as well as 2011 Pmic increases with increasing soil moisture and clay content as well as NaOH-Po, HCl-P, H_2SO_4-P, Σ $NaHCO_3$-Po + NaOH-Po, total P, Corg, total N, Nmic, Cmic and phosphatase and decreases with increasing sand content as well as $NaHCO_3$-Pi and $NaHCO_3$-Po.

Only for soil temperature an oppositely directed trend on Pmic was observable interannually: a positive correlation to Pmic was found in 2011 and a negative correlation to Pmic in 2014. Plots, for which Pmic and soil temperature data from 2011 as well as 2014 were available corroborate this opposite trend (2011: $r = 0.32$, $P < 0.001$, $n = 60$; 2014: $r = -0.31$, $P < 0.05$, $n = 60$). Therefore, it could not be explained by the sample size or the sampled plot. However, soil temperature was significantly higher in 2011 than in 2014 ($P < 0.05$) (**Supplementary material: Table S 3**).The correlation of air temperature to Pmic was not significant for 2011 ($r = -0.07$, $P = 0.53$, $n = 80$) and shows the same decreasing effect for 2014 as soil temperature (**Table 3.3**). Although, soil temperature and air temperature show a positive correlation (2011: $r = 0.64$, $P < 0.001$, $n = 81$, 2014: $r = 0.61$, $P < 0.001$, $n = 119$), which was expectable, finding the mechanism leading to the observed interannual trend is a rather tough task to complete.

But generally, (soil) moisture has a higher influence on microbial biomass, i. e. Pmic, than (soil) temperature (Donnelly et al. 1990, Grayston et al. 2001, Khan and Joergensen 2006, Regan et al. 2014). However, the observed decreasing Pmic concentration from 2011 to 2014 contrasted with increasing soil moisture. Therefore, probably either an unmeasured variable or a combination of a variety of nonlinear factors led to the observed result, as the same analytical and statistical method was used for determination of Pmic and calculated blanks were zero.

Disentangling the driving factor for Pmic (Model 1)

The complex interactions among the correlated variables controlling Pmic in grassland soils associated with the limitations associated with bivariate correlations call for SEMs to be performed for the data of 2014 as well as 2011.

For 2014, the prerequisites for a PCA to reduce the number of variables in a SEM were fulfilled (KMO = 0.26, Bartlett $P < 0.001$). The PCA resulted in three components explaining 69.4 % of the variance in the data. Based on the highest loading in each component, following variables were selected: HCl-P (Component 1; loading 0.89), NaOH-Pi (Component 2; loading 0.83), total C (Component 3; loading 0.78). HCl-P concentrations were replaced by the variable with the second-highest loading in Component 1, i. e. total P concentrations (loading 0.85), for a point-by-point comparison between the years. Hence, the SEM for 2014 included the same variables as described for 2011 (**Section 3.1**).

Model 1 was used with Corg as the driving factor influencing diversity and Pmic (for explanation see **section 3.1**). The SEMs showed an adequate fit between data and model (**Table 3.4**) with a mean

explained variance of Pmic of 34 % (median = 26 %) for 2011 and 45% (median = 34 %) for 2014 (**Figure 3.5**).

Table 3.4: Output of each structural equation model 1 on the relationships between different abiotic controlling factors, plant diversity and Pmic (ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin).

Model	Model 1 (2011)				Model 1 (2014)			
	χ^2	df	probability level	RMSEA index	χ^2	df	probability level	RMSEA index
grassland soils								
all regions	1.98	1	0.159	0.081	3.21	1	0.073	0.122
ALB	0.01	1	0.975	< 0.001	< 0.01	1	0.967	< 0.001
HAI	0.86	1	0.353	< 0.001	0.90	1	0.343	< 0.001
SCH	0.01	1	0.931	< 0.001	0.07	1	0.790	< 0.001

Comparing 2011 and 2014 there are some variations (**Figure 3.5**). In general, the explained variance of Pmic increased within the three exploratories prominently (**Figure 3.5**), only for all regions it decreased (Grass 2011 = 30 %, Grass 2014 = 15%). The explained variance of diversity forfeits for all models 2014, Corg is more or less stable. For all regions (Grass 2011 and 2014) diversity influences Pmic significantly, for each region individually it is not significant anymore except for AEG 2014. The influencing effect of Corg increased in 2014 compared to 2011. Merging all regions, the influencing effect of Corg in 2011 showed a highly positive correlation and for 2014 it showed a low negative correlation. In SCH Corg becomes a positive influencing factor of Pmic in 2014. In SCH 2014 total P and Σ $NaHCO_3$-Pi + NaOH-Pi had the highest influence on Pmic of the measured variables in comparison to all other driving models. In general, the model for 2014 corroborates Corg as the most driving factor for Pmic. An explanation for the increasing influence if Corg on Pmic could be that Pmic compared to the years decreased stronger (and significant) than Corg (Corg 2011 (Schöning and Trumbore 2014): AEG = 65.1 [±2.0] mg kg^{-1}, HEG = 45.6 [±1.8] mg kg^{-1}, SEG = 105.8 [±14.3] mg kg^{-1}; Corg 2014 (Schöning et al. 2015): AEG = 64.3 [±1.9] mg kg^{-1}, HEG = 37.7 [±1.4] mg kg^{-1}, SEG = 103.8 [±13.8] mg kg^{-1}). Only in SCH Pmic seems to be unaffected by Corg. Comparing Corg and Pmic concentrations it has to be noticed that Corg in SCH is much higher than ALB and HAI otherwise than Pmic (**Figure 3.4**).

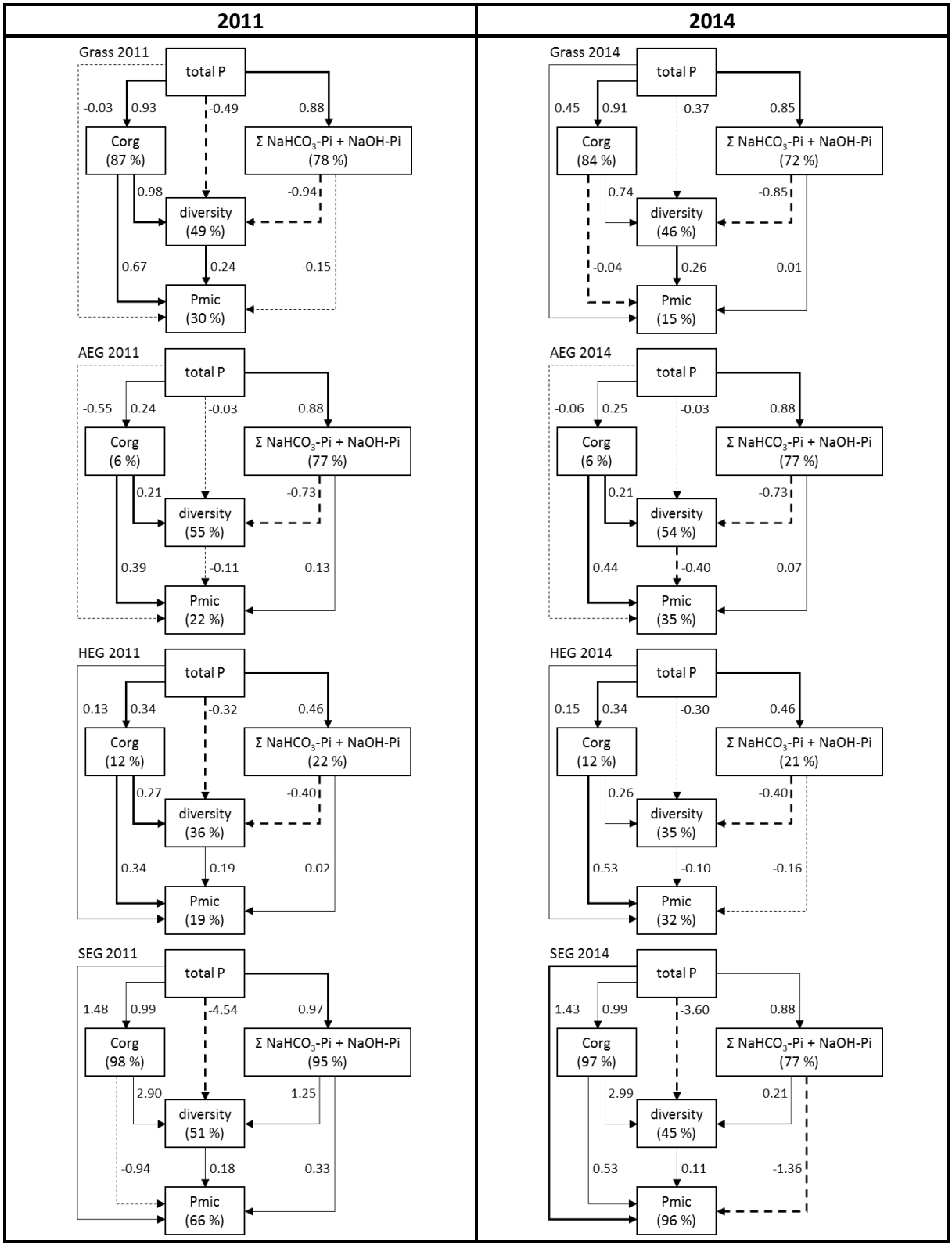

Figure 3.5: Structural equation models of the relationships between different abiotic controlling factors, plant biodiversity (diversity) and microbial phosphorus (Pmic, year = 2011 and 2014) in grassland sites with organic carbon (Corg) as driving factor for all three regions together (Grass), Schwäbische Alb (AEG), Hainich-Dün (HEG) and Schorfheide-Chorin (SEG) (percentage of explained variance of the variable in brackets; numbers on arrows: standardized path coefficients; path lines: bold lines: $P < 0.05$, small lines: $P \geq 0.05$, solid line: positive path coefficient, broken line: negative path coefficient).

Parallel to the increasing influence of Corg on Pmic a decreasing effect of Σ $NaHCO_3$-Pi + NaOH-Pi could be measured. It seemed that influence of Corg eliminated influence of P. But this fact could not be validated because Corg concentrations were measured within the same sample as Pmic (snap-shot measurements), whereas P concentrations were measured before. A repeated measurement of P fractions, which is possible to air-dried samples, could be helpful for an explanation in detail. As well as the influence of Σ $NaHCO_3$-Pi + NaOH-Pi the influence of diversity decreased. Therefor a repeated capture of diversity closer to the sampling time/year could be mandatory. There was no change on influence of total P to Σ $NaHCO_3$-Pi + NaOH-Pi because for 2014 the same data set as 2011 was used.
The conclusion of disentangling the driving factor is that Corg seemed to be most important and compared to 2011 Corg did not decrease to 2014 as Pmic. But remeasurements of P fractions and diversity could overturn this statement.
Therefore, by means of this data no advice for a P conservation management is possible and Pmic should be regulated via influencing Corg and/or diversity. However, soil microbial biomass increases with increasing species richness (Eisenhauer et al. 2010) and there is an additional positive effect of plant species richness on P exploitation (Oelmann et al. 2011), which only could evidence for the forest sites (**Table 3.1**, **Figure S 1**, **Figure S 2**). For grassland sites there are positive but non-significant correlations (Pmic × diversity: 2011: $r = 0.14$, $P = 0.10$; 2014: $r = 0.07$, $P = 0.41$).

Influencing management effects

Pmic concentrations are highly variable spatially (Bliss et al. 2004, Cole et al. 1977, Helal and Dressler 1989) as well as temporally (Baum et al. 2003, Buchanan and King 1992, Chen et al. 2003, Díaz-Raviña et al. 1995) but neither fertilization, management nor the number of cuts per year seemed to influence Pmic in 2011 (**Table 3.2**). For 2014 one effect for HAI (differences influenced by number of cuts per year) and one effect for SCH (differences influenced by management) could be measured (Table **3**.**5**).

Table 3.5: Mean [± standard error] of Pmic concentrations 2014 differentiated to region and land-use type (in mg kg^{-1}; na = not available, different letters present significant differences within the exploratory (region) [P <0.05]).

	land-use type	region					
		Schwäbische Alb		Hainich-Dün		Schorfheide-Chorin	
grassland soils 2014	fertilization						
	yes	49.9 [±3.7]	a	36.2 [±4.3]	a	16.7 [±7.4]	a
	no	53.8 [±5.1]	a	37.8 [±3.2]	a	24.7 [±3.8]	a
	management						
	meadow	53.7 [±5.2]	a	29.1 [±3.8]	a	34.4 [±8.6]	a
	mown pasture	47.4 [±6.4]	a	39.6 [±4.7]	a	21.2 [±5.9]	ab
	pasture	52.2 [±5.1]	a	37.0 [±3.8]	a	17.7 [±3.8]	b
	number of cuts per year						
	0	48.9 [±5.5]	a	32.7 [±3.0]	b	16.1 [±3.1]	a
	1	60.6 [±7.9]	a	38.4 [±3.6]	ab	23.6 [±6.4]	a
	2	52.7 [±3.5]	a	68.3 [±17.7]	a	36.3 [±9.9]	a
	3	50.2 [±10.9]	a	22.3 [±4.6]	b	29.0 [±23.9]	a

The negligible fertilization effect could be explained with lower P fertilization rates. P fertilizer was used rarely: March 2011: AEG18 and AEG14, April 2014: SEG08, SEG23, SEG26 (Gockel and Weisser 2017). All other plots were only fertilized with N (Gockel and Weisser 2017).

Only plots used as meadow or mown pasture were managed via fertilization, because pasture are more or less balanced in P budget (Traub 2016). This seemed to be true for all exploratories in 2011 (**Table 3.2**) as well as ALB and HAI in 2014 but not for SCH in 2014 (Table **3**.**5**). Here, pasture had a negative effect on Pmic concentrations in soil. This trend of lower Pmic in SCH pasture was not new. Also, 2011 Pmic in SCH pasture was lower than SCH meadow and SCH mown pasture (**Table 3.2**) but not significant. May be the farmers prevented P losses via fertilization (Gockel and Weisser 2017) or the rhythm of the grazing animals changed. It was able to relate to the grazing animals and time, but there was no information about additional fodder. In case the grazing animals got some additional fodder, the grazing effect was not as strong as without.

The detected effect of cutting in HAI could result in the repetition. At the beginning (**Table 3.2**) the loss of P could be compensated, so the effect of cutting once, twice or trice should not as effective as after years (Table **3**.**5**).

Soil moisture depending on precipitation regulates substrate availability, soil pH and temperature (Harris 1981). The stability of Po in soil to mineralization decreased as soil pH increased (Greb and Olsen 1967). So Pmic might be dependent on pH as well as Po. Both differentiate between the three exploratories and are influenced by different land use (Alt et al. 2011). Therefore, effects of management measures on Pmic concentrations in grassland soils are mediated through direct P input or effects such as changes in pH (Ayaga et al. 2006). If Histosols in SCH were excepted, regions with high annual precipitation, high Corg concentrations in soil, and high soil pH are characterized by highest

Pmic concentrations in soil (ALB > HAI > SCH). Soil moisture positively affects organic matter concentrations and microbial activity in soil (Frossard et al. 1989, Kieft et al. 1987, Liu et al. 2010, Lynch and Whipps 1990) and Corg could be identified as driving factor for Pmic (**Figure 3.5**, **Figure 3.2**). Soil pH is Major factor for variation in P studies (Alt et al. 2011, Cole et al. 1977, Heinze et al. 2010). Fertilization and land-use management interfere with the P cycle too (Alt et al. 2011, Heinze et al. 2010). P Fertilization has an increasing effect on Pmic (Liu et al. 2008) and there is a positive effect on P uptake by fertilization (Kuo et al. 2005). Mineral fertilizer applications decrease soil pH (Heinze et al. 2010) as indirect effect of management. To dissolve P containing minerals soil pH must drop below 7.5, then the dissolved PO_4 can be used by plants, leached from the system or precipitated as calcium (Ca), aluminium (Al) or iron (Fe) PO_4 (Smeck 1973). Liming adjusts the pH to 5.5 – 5.6. Thus, the concentrations of soluble and exchangeable Fe and Al will be reduced, because they react with the available PO_4 to form poorly soluble Al and Fe PO_4 (Haynes 1982). Nevertheless, effect of management on Pmic is not as strong as expected but also measured for microbial community within the exploratories (Herold et al. 2014b). The fertilization application rate of P is negligible and fertilization at the study site of the Exploratories does not include liming, as mostly N fertilizer was applied (Gockel and Weisser 2017). The two “snapshot” measurements of Pmic differ between the years. But there are no strong differences depending on land use and management (2011: **Table 3.2**; 2014: Table **3.5**) nor on influencing factors (**Figure 3.5**).

3.2.2 Microbial phosphorus influenced by soil type

Corg, which was disentangled as the most driving factor (**section 3.1.2**, **3.2.1**), depends on soil type (Hartemink and McSweeney 2014). Regarding the influence of soil type on Pmic for ALB and HAI no significant differences could be detected (Table **3.6**). Only in SCH the Histosol stood out (Table **3.6**). Generally, Histosols are rich in Corg and provided with high soil moisture (Osman 2013b) and only if there is less resin-P, Histosols show high phosphatase activity (Baum et al. 2003). Furthermore, as explained in **section 3.1**, fine-textured soils contain more microbial biomass than coarse-textured soils (Naveed et al. 2016). This could explain the higher Pmic contents in Histosols as it establishes a link to the variables controlling Pmic (**Table 3.3**) in grassland soils and known properties of soil types. Indeed, there are also differences in soil moisture, Corg and texture within other soil types but Histosols show higher extremes (Osman 2013b, Hartemink and McSweeney 2014).

Table 3.6: Mean [± standard error] of Pmic concentrations 2011 and 2014 differentiated to region and soil (in mg kg^{-1}; na = not available, different letters present significant differences within the exploratory (region) [P <0.05]).

	soil type	region					
		Schwäbische Alb		Hainich-Dün		Schorfheide-Chorin	
grassland soils 2011	Albeluvisol	na		na		26.7 [±4.0]	b
	Cambisol	56.6 [±6.7]	a	47.6 [±3.7]	a	24.1 [±1.6]	b
	Gleysol	na		na		46.8 [±12.9]	b
	Histosol	na		na		72.0 [±6.5]	a
	Leptosol	65.3 [±4.4]	a	na		na	
	Luvisol	na		na		16.5 [±0.8]	b
	Stagnosol	na		59.6 [±4.6]	a	na	
	Vertisol	na		48.1 [±14.9]	a	na	
grassland soils 2014	Albeluvisol	na		na		7.2 [±0.9]	b
	Cambisol	56.6 [±6.4]	a	35.4 [±3.7]	a	11.5 [±1.9]	b
	Gleysol	na		na		24.2 [±6.1]	ab
	Histosol	na		na		44.9 [±6.8]	a
	Leptosol	49.5 [±3.5]	a	na		na	
	Luvisol	na		na		9.7 [±1.2]	b
	Stagnosol	na		42.4 [±4.5]	a	na	
	Vertisol	na		28.4 [±4.3]	a	na	

Histosols are characterized by high contents of total P (Baum et al. 2003, Schlichting et al. 2002), low contents of bioavailable P (Baum et al. 2003) and high Corg contents (Andreux 1996, Eswaran et al. 1993, Osman 2013a). For Histosols in SCH total P contents are not available (Alt et al. 2011), but measurements show higher Corg contents (2011: Schöning and Trumbore (2014) as well as 2014: Schöning et al. (2015)). Comparing all soil types in 2011 Histosols had the lowest resin-P contents (Albeluvisol = 29.9 [± 3.6] mg kg^{-1}, Cambisol = 21.6 [± 2.9] mg kg^{-1}, Gleysol = 16.1 [± 5.1] mg kg^{-1}, Histosol = 10.6 [± 2.0] mg kg^{-1}, Luvisol = 22.2 [± 3.1] mg kg^{-1}). In 2014 the resin-P contents in Gleysol were the lowest (Albeluvisol = 37.0 [± 3.0] mg kg^{-1}, Cambisol = 27.6 [± 4.3] mg kg^{-1}, Gleysol = 14.4 [± 4.8] mg kg^{-1}, Histosol = 25.6 [± 3.9] mg kg^{-1}, Luvisol = 32.5 [± 6.2] mg kg^{-1}). Additionally, determined Histosols in SCH have a high clay content (Schöning et al. 2011) and showed higher phophatase activity (2011: Kandeler et al. (2017a); 2014: (Kandeler et al. 2017c)) than other soil types in SCH. So, the hypothesis that Pmic depending on soil type could be validate for Histosols only.

3.3 Quantification of enzyme-mediated hydrolysis by means of the oxygen isotope ratio of phosphate in soil

The identification of reactions involved in Pi release in soil could help optimizing the nutrition of organisms particularly in the view of imminent supply shortages of mineral P fertilizer. This study presents an approach based on $\delta^{18}O$ values of resin-extractable Pi ($\delta^{18}O_{Pi}$) in soil. This approach relies on the incorporation of O atoms of ambient water into PO_4 that results from enzymatic activity. Therefor soil samples were incubated with waters differing in the O isotope composition and used a linear regression of $\delta^{18}O_{Pi}$ values on $\delta^{18}O_{SW}$ values in soil water to quantify the total biological O incorporation into Pi during incubation. Total biological O incorporation into Pi released during incubation increased with time from 5 % day one to 36 % at day 14 calling for cautious interpretations of potential enzyme activities. Irrespective of incubation time, enzymatic hydrolysis associated with equilibrium fractionation dominated (> 90 % of the total biological O incorporation). Calculations to distinguish kinetic from equilibrium fractionation processes yielded reliable results with a good precision (coefficient of variation < 10 %) and only small deviations (< 5 %) in a sensitivity analysis. This approach offers novel insights into actual enzymatic processes involved in Pi release in soil. All values are given as mean [± standard error] unless stated otherwise.

3.3.1 Total biological O incorporation into Pi

Compared to background ($\delta^{18}O_{SW/t0}$), $\delta^{18}O_{SW/ti}$ values of all labeling treatments were significantly different (t-test: 0.001 < *P* < 0.05). However, the difference between background and the labeling treatment based on ^{18}O-depleted water (L: $\delta^{18}O_W$ = -33 ‰) was very small (averaged across incubation 0.6 ± standard error 0.1 ‰, **Supplementary material**: **Table S 5**) and close to the limit of detection (see **Section 2**). The effect of labeling soil water by using ^{18}O-depleted water in this study was likely too small to translate into processes incorporating O atoms from ambient water such as the release of Pi by biological processes. If both a negligible increase in soil moisture (< 15 %, see **Section 2**) and a detectable difference between $\delta^{18}O_{SW/t0}$ and $\delta^{18}O_{SW/ti}$ values are aimed at, highly ^{18}O-depleted water obtained e.g. in polar regions (Bowen 2010, Ruppenthal et al. 2010) must be used.

Nevertheless, the O isotopic signature of soil water ($\delta^{18}O_{SW}$) significantly differed depending on $\delta^{18}O$ values of added water (**Figure 3.6**a, treatment effect in repeated measures ANOVA: *P* < 0.001). $\delta^{18}O_{SW}$ values were constant during incubation irrespective of labeling treatment (**Figure 3.6**a) as corroborated by non-significant time (*P* = 0.17) as well as time × treatment interaction effects (*P* = 0.18). Due to water present in field-fresh soil, the efficiency of isotopic labeling (mean shift during the incubation of +46.6 ± 0.7 ‰ and +69.1 ± 0.6 ‰ comparing $\delta^{18}O_{SW/ti}$ and $\delta^{18}O_{SW/t0}$ values in the M

and H treatments, respectively; **Supplementary material**: **Table S 5**) was smaller than those achieved by using the isotopically labeled water as a medium in culture studies (Chang and Blake 2015, Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015).

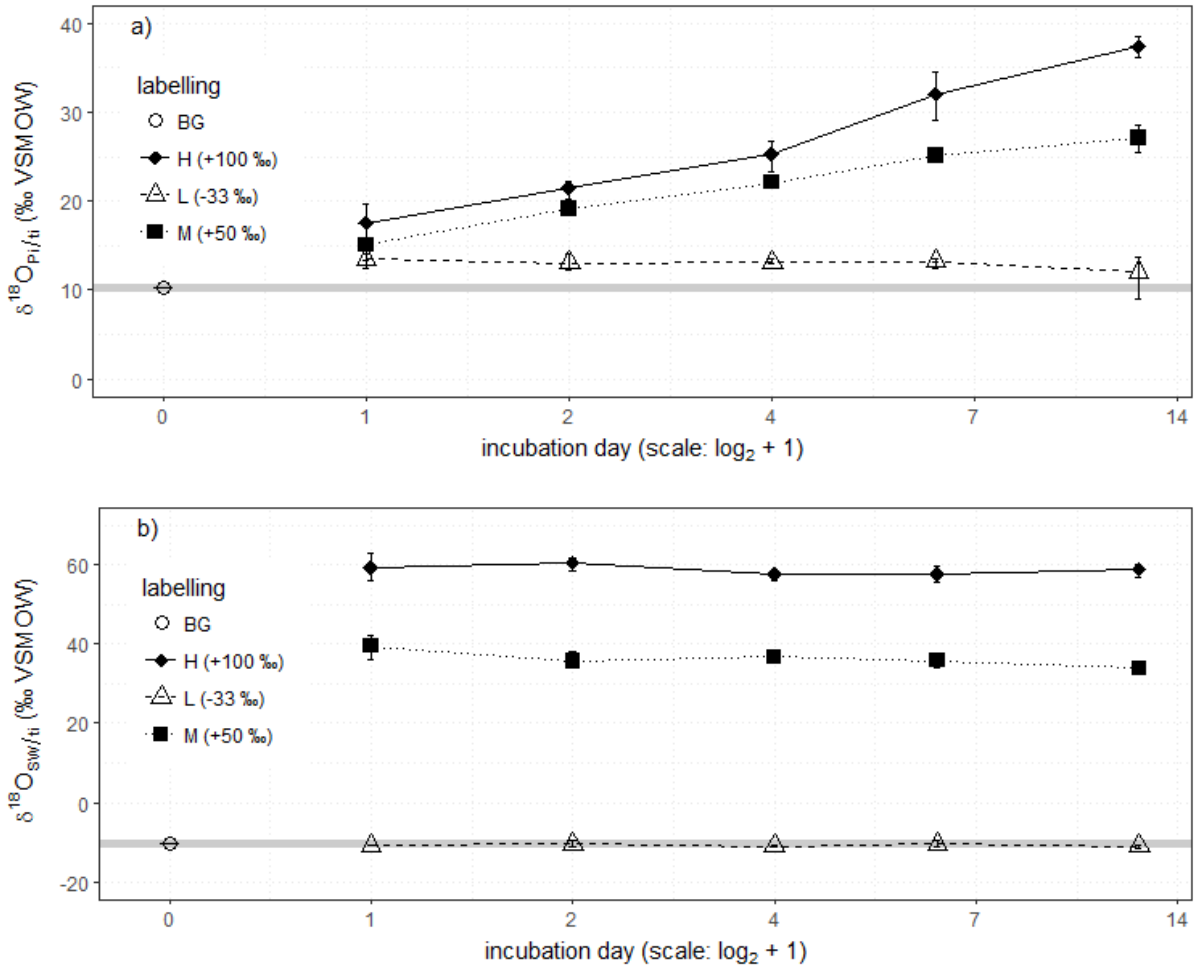

Figure 3.6: Temporal course of (a) $\delta^{18}O$ values in soil water at time point ti ($\delta^{18}O_{SW/ti}$), and (b) $\delta^{18}O$ values of resin-extractable phosphate at time point ti ($\delta^{18}O_{Pi/ti}$) depending on the labeling treatment (isotopic signature of the water added to the soil $\delta^{18}O_W$ = +100 ‰ [diamonds]; +50 ‰ [quadrats]; -33 ‰ [rectangles]). Background (BG) $\delta^{18}O_{SW}$ and $\delta^{18}O_{Pi}$ values before labeling (t0) are displayed as open circles. Each symbol represents the mean of three replicate measurements (in some cases whiskers referring to the standard error are smaller than the symbols). The grey bar is indicative of mean ± standard error of $\delta^{18}O_{SW/t0}$ and $\delta^{18}O_{Pi/t0}$ values and is extended for the whole incubation period to ease the visualization of differences to BG values at a given point in time.

Oxygen atoms from isotopically labeled soil water were incorporated into Pi released during incubation as indicated by significant differences between $\delta^{18}O_{Pi/t0}$ and $\delta^{18}O_{Pi/ti}$ values at all times and for all treatments (**Figure 3.6**b, matched pairs t-test, $P < 0.001$). There were only a few exceptions to this general pattern (matched pairs t-test $P > 0.05$ for treatment M [$\delta^{18}O_W$ = +50 ‰] at days one and two and treatment L [$\delta^{18}O_W$ = -33 ‰] at day 14). Again, differences in $\delta^{18}O_{Pi}$ values between background and the L treatment were very small (0.3 ± 0.3 ‰) and close to the limit of detection (see **Section 2.5**). Labeling treatments significantly differed in $\delta^{18}O_{Pi/ti}$ values at all times (significant treatment effect in

repeated measures ANOVA: $P < 0.001$) except for day one (univariate ANOVA: $P = 0.12$). No treatment effects on $\delta^{18}O_{Pi/ti}$ values one day after labeling might be explained by either i) negligible biological Pi release and accordingly, negligible total biological O incorporation after just one day of incubation, ii) rapid uptake of biologically released and thus, isotopically shifted Pi by microorganisms, or iii) an initial compensation between the shift induced by labeled soil water (ca. +40 and +70 ‰) and by kinetic fractionation factors (minimum of -38 to -39 ‰, (Liang and Blake 2006b, von Sperber et al. 2014)) or by a combination of these. Because of physicochemical (desorption), geochemical (dissolution), and biological processes (enzymatic hydrolysis and cell lysis) potentially contributing to Pi release at a given point in time (**Figure 1.4**), explanation i) cannot be excluded. However, as resin-extractable Pi concentrations remained constant during the first seven days of incubation (repeated measures ANOVA: non-significant time or time × treatment interaction effects, $P > 0.29$; **Supplementary material: Table S 5**), and as other P pools such as loosely sorbed Pi was shown to rapidly equilibrate with microbially overprinted Pi (Jaisi et al. 2011), this study assumed that processes underlying Pi release did not change over time. Therefore, this study considered it more likely that the ratio between microbial P immobilization and Pi release remained constant and thus, that biological Pi release was small one day after labeling (explanation i). In microorganisms $\delta^{18}O_P$ values ($\delta^{18}O_{Pmic}$) were not measured. But even if hadith ab been, it would not be able to follow the fate of Pi taken up by microorganisms, as $\delta^{18}O_{Pmic}$ values would have been influenced by cell-internal P cycling including exchange reactions with cell water carrying itself the isotopic label (deriving from isotopically labeled soil water). Finally, kinetic fractionation might play a role which is discussed in the respective chapter (**Section 3.3.2**) also shedding light on the two previous possibilities.

The significant interaction between time and treatment (repeated measures ANOVA, $P < 0.001$) if analyzing the temporal course of $\delta^{18}O_{Pi/ti}$ values illustrates the discrepancy between the treatments based on either ^{18}O-depleted water (L) and ^{18}O-enriched water (M, H). $\delta^{18}O_{Pi/ti}$ values of the L treatment did not change whereas $\delta^{18}O_{Pi/ti}$ values increased over time in the M and H treatments (**Figure 3.6**b) as indicated by a significant effect of time in a repeated measures ANOVA ($P < 0.001$) with a log-linear shape of the contrast ($P < 0.001$). As $\delta^{18}O_{SW}$ values were constant during incubation (**Figure 3.6**a), this increase in $\delta^{18}O_{Pi/ti}$ values of M and H treatments reflects an increasing incorporation of O atoms from isotopically labeled soil water into Pi released during incubation. Similarly, Mediterranean soils with isotopically labeled water ($\delta^{18}O$ of water = +99.8 ‰ ≈ $\delta^{18}O_{SW}$) set near field capacity, Gross and Angert (2015) found increasing $\delta^{18}O_{Pi/ti}$ values during a 14-day incubation. However, the increase in $\delta^{18}O_{Pi/ti}$ between day 0 and day 14 was smaller (< 10 ‰) as compared to this study (> 20 ‰, **Figure 3.6**). This illustrates that the incubation conditions (e. g. incubation temperature of 20 °C in this study vs. 30 °C in the study of Gross and Angert (2015); field-fresh vs. dried soil; soil moisture nearly unchanged vs. set to field capacity, respectively) must be chosen cautiously to guarantee comparable results if using

soil samples from different sites or studies. Alternatively, the difference might reflect different site conditions (pH, Corg, P fractions, enzyme/microbial activity, etc.) calling for further studies based on a consistent approach as used in this study.

If quantifying the total biological O incorporation into released Pi using the regression of $\delta^{18}O_{Pi/ti}$ on $\delta^{18}O_{SW/ti}$ values, significantly higher O incorporation factors *a* were found towards the end as compared to earlier periods of the incubation (**Figure 3.7**, significant effect of time in repeated measures ANOVA, $P < 0.001$).

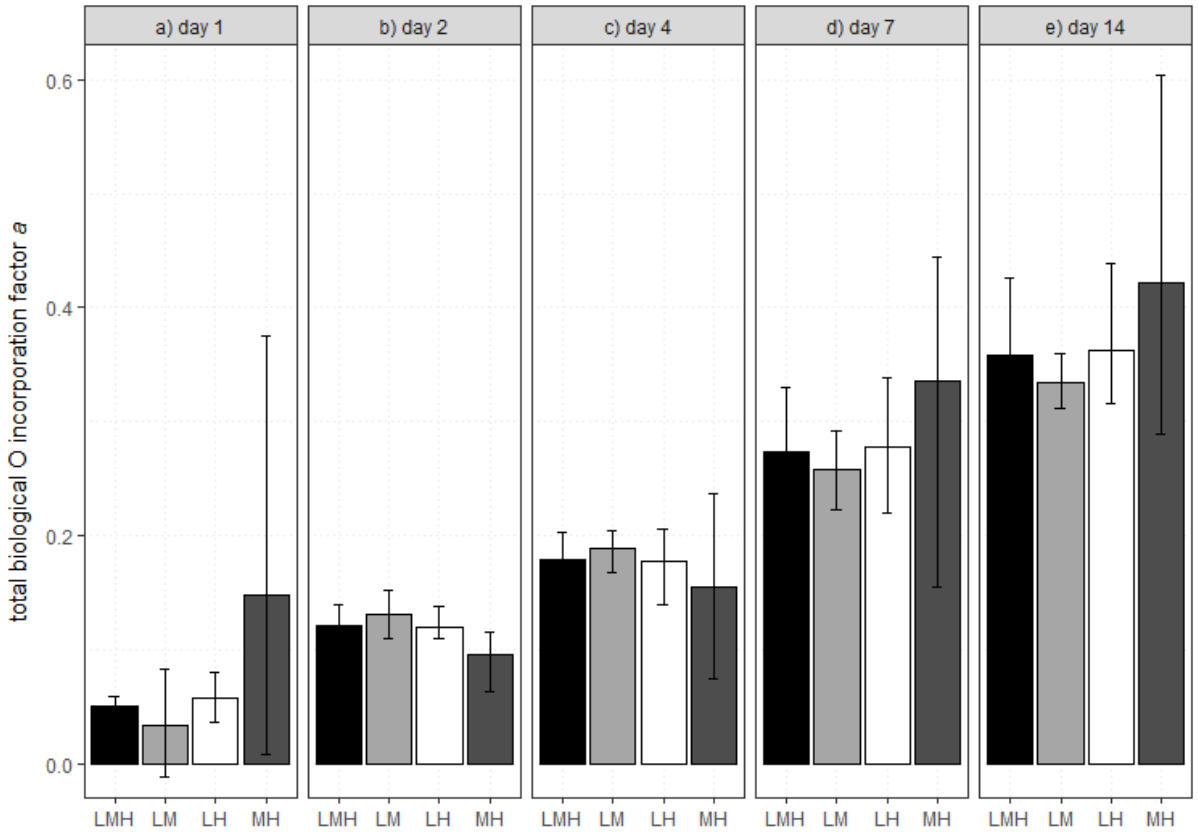

Figure 3.7: The total biological O incorporation factor *a* calculated as the slope of the regression of $\delta^{18}O_{Pi/ti}$ on $\delta^{18}O_{SW/ti}$ values for each point in time (ti) based on the combination of the labeling treatments (L = low [-33 ‰], M = medium [+50 ‰], H = high [+100 ‰] with $\delta^{18}O_W$ values in squared brackets) after one (a), two (b), four (c), seven (d), and 14 days (e) of incubation. Each bar represents the mean of three replicate measurements with whiskers referring to the standard error. The total biological O incorporation factor *a* indicates the proportion of O atoms in phosphate molecules after incubation that have experienced biological incorporation of O atoms from ambient water (by processes associated with kinetic and/or equilibrium fractionation).

Initially lower total biological O incorporation might be related to reaction kinetics associated with the enzymes involved. For PPase associated with equilibrium fractionation and a sequential incorporation

of all four O atoms in a PO_4 molecule, two studies reported that steady state conditions (= slope of ca. 1 of the regression of $\delta^{18}O_{Pi/ti}$ on $\delta^{18}O_W$ values equal to incorporation of all four O atoms) were reached between 1 and 3 days (Blake et al. 2005, Chang and Blake 2015). However, in contrast to studies based on isotopically labeled water, enzyme/microorganisms, and Po substrates in the laboratory (Blake et al. 2005, Chang and Blake 2015, Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015), the total biological O incorporation factor *a* in this study comprises not only the proportion of O atoms incorporated relative to all four O atoms in one phosphate molecule but, in addition, the proportion of PO_4 molecules itself that has experienced O incorporation during incubation. This means that an "untouched" proportion of PO_4 molecules that were either already present before incubation or derive from desorption/dissolution processes not associated with O incorporation from ambient water has to be accounted for (**Figure 1.4**).

3.3.2 Disentangling the role of isotopic equilibrium and kinetic fractionation

First, it was assumed that O incorporation associated with equilibrium fractionation dominated. Therefore, a simplified two-end-member mixing model was used including a proportion of molecules that did not experience O incorporation from labeled soil water (1 – *a*) and a proportion of molecules that experienced O incorporation associated by equilibrium fractionation (*a*) for each of the treatments **(Equ. 3.1)**:

$$\delta^{18}O_{Pi/ti}{}' = (1-a)\cdot\delta^{18}O_{Pi/t0} + a\cdot\delta^{18}O_{Pi/Equ} \quad \text{(Equ. 3.1)}$$

with $\delta^{18}O_{Pi/ti}$' as the expected $\delta^{18}O$ value of Pi at a given point in time (i), $\delta^{18}O_{Pi/t0}$ as the initial (t = 0) $\delta^{18}O$ value of Pi, $\delta^{18}O_{Pi/Equ}$ as the $\delta^{18}O$ value of Pi deriving from equilibrium fractionation (calculated after Chang and Blake (2015), and *a* derived from the three point [LMH] regression).

Then the measured $\delta^{18}O_{Pi/ti}$ on the expected $\delta^{18}O_{Pi/ti}$' values were regressed. For the L treatment, all values clustered close to the 1 : 1 line (mean ± standard error of deviation = -0.01 ± 0.32) as there was no temporal shift in neither $\delta^{18}O_{Pi/ti}$, $\delta^{18}O_{SW/ti}$ (**Figure 3.6**a, b), nor in according $\delta^{18}O_{Pi/ti}$' values. If alternatively the fractionation factor resulting from equilibrium fractionation (Chang and Blake 2015) was calculated, again a close match between observed and expected 1000 ln $\alpha_{(PO4\text{-}H2O)}$ (23.74 ± 0.38 and 23.68, respectively) at all time steps of the incubation in the L treatment was found. As $\delta^{18}O_{Pi/ti}$ values were close to background $\delta^{18}O_{Pi/t0}$ values at all times, the small deviation from the 1 : 1 line suggests that for the L treatment biological O incorporation associated with equilibrium fractionation dominated already before and subsequently also during the incubation. Therefore, biological

processes releasing Pi did not differ before and during the incubation corroborating the assumption of steady-state conditions (see Blake et al. (2005), Gross et al. (2015)). For the M and H treatments, a close relationship was found between expected ($\delta^{18}O_{Pi/ti}$') and observed $\delta^{18}O_{Pi/ti}$ values based on the simplified assumption of the sole contribution of equilibrium fractionation to biological O incorporation (M: $\delta^{18}O_{Pi/ti} = 0.96 \times (\delta^{18}O_{Pi/ti}')$, $r = 0.86$, $P < 0.001$; H: $\delta^{18}O_{Pi/ti} = 0.99 \times (\delta^{18}O_{Pi/ti}')$, $r = 0.99$, $P < 0.001$). Thus, the observed $\delta^{18}O_{Pi/ti}$ values were lower as expected and 25 % of the variation in observed $\delta^{18}O_{Pi/ti}$ values remained unexplained by expected $\delta^{18}O_{Pi/ti}$' values for the M treatment.

Second, therefore it was tried to further improve the prediction for all treatments by acknowledging that the O incorporation factor *a* must be decomposed in two components (**Figure 1.4**), i. e. a proportion of kinetic fractionation (*c*) and of equilibrium fractionation (*a* – *c*). Thus, the $\delta^{18}O_{Pi}$ values at a given point in time during incubation ($\delta^{18}O_{Pi/ti}$) can be attributed to Pi released associated with kinetic and equilibrium and a proportion of phosphate molecules that were not released by enzymatic hydrolysis (see **Figure 1.4** and **(Equ. 1.4)** in **section 1**). This equation can be rearranged to solve for *c* **(Equ. 3.2)**:

$$c = \frac{\delta^{18}O_{Pi/ti} - (1-a) \cdot \delta^{18}O_{Pi/t0} - a \cdot \delta^{18}O_{Pi/Equ}}{\delta^{18}O_{Pi/kin} - \delta^{18}O_{Pi/Equ}} \quad \text{(Equ. 3.2)}$$

with $\delta^{18}O_{Pi/Equ}$ and $\delta^{18}O_{Pi/kin}$ calculated according to **(Equ. 2.5)** and **(Equ. 2.6)**, respectively. Please note that mean values ($\delta^{18}O_{Po}$ = 15 ‰, $\varepsilon_{Pi/W}$ = -15 ‰) published in the literature so far (Liang and Blake 2006b, von Sperber et al. 2014, 2015) were used for $\delta^{18}O$ values of Po and the kinetic fractionation factor (but see **Section 3.3.3** as well). On average, *c* was small, i. e. contributing less than 10 % to total O incorporation in most cases, irrespective of the treatment used for calculation (**Table 3.7**) which could be expected based on the results of the simplified mixing model presented above. There was no clear temporal trend in *c* during incubation (repeated measures ANOVA, $P > 0.05$).

Table 3.7: Mean kinetic O incorporation factors *c* calculated based on (Equ. 3.2) during incubation for each treatment with the contribution of the kinetic fractionation factor *c* to the total O incorporation factor *a* (*c*/*a* × 100, %) given in parentheses.

treatment	day of incubation				
	1	2	4	7	14
L (-33 ‰)	0.00 (0)	0.01 (12)	0 (0)	0.03 (7)	0.18 (30)
M (+50 ‰)	0.01 (14)	0.00 (1)	0.01 (2)	0.03 (8)	0.06 (10)
H (+100 ‰)	0.00 (0)	0.00 (0)	0.01 (6)	0.09 (22)	0.10 (18)

Again, this indicates that processes were at steady-state conditions meaning that the ratio between processes associated with equilibrium fractionation (e. g. active or passive release of Pi by/from microorganisms; Blake et al. (1997), (2005), Chang and Blake (2015)) to processes associated with kinetic fractionation (e. g. Pi release by extracellular enzyme activity; Liang and Blake (2006b), (2009), von Sperber et al. (2014), (2015)) did not change during the incubation. Accordingly, this study inferred that the small differences in $\delta^{18}O_{Pi/t1}$ among treatments at day one (**Figure 3.6**) were not due to a compensation of the isotopic shift to positive $\delta^{18}O_{Pi}$ values by incorporation of ^{18}O-enriched water and the negative shift induced by enzyme activity (which would have required the predominance of kinetic over equilibrium fractionation). Similarly, several indications of steady-state conditions during incubation render the explanation of pronounced uptake of isotopically shifted Pi at day one unlikely. Instead, the initially small total biological O incorporation factor *a* was likely caused by a small biological Pi release as compared to the amount of phosphate molecules already present constraining the extent of the shift in $\delta^{18}O_{Pi}$ values one day after labeling.

For the calculation of *c*, it was necessary to constrain thresholds in which *c* yielded defined results. Therefore, values of $c < 0$ and $c > a$ were set to 0. This step was necessary for $n = 13$, 7, and 6 out of 18 cases in the L, M, and H treatment, respectively. The reason for these non-defined values originate from $\delta^{18}O_{Pi/ti}$ values that were slightly higher as could be expected based on the simplified two-end member mixing model (= numerator of **(Equ. 3.2)**; range of +0.1 to +2.4 ‰). In these cases, *c* was < 0 as the denominator ($\delta^{18}O_{Pi/kin} - \delta^{18}O_{Pi/Equ}$) was negative (whereas both numerator and denominator were negative in all other cases). A positive shift of $\delta^{18}O_{Pi}$ values from those to be expected by equilibrium fractionation in other studies was explained by fractionation associated with uptake of phosphate by microorganisms leaving $\delta^{18}O_{Pi}$ values of phosphate remaining in (soil) solution ^{18}O enriched (Blake et al. 2005, Gross et al. 2015). As no effect of microbial uptake of Pi in soil has been reported as well (Gross and Angert 2015) and in the light of reproducibility and accuracy issues ($\delta^{18}O_{Pi/ti}$ values of M and L treatment at the upper limit of standards used for calibration), these shifts should not be over-interpreted.

Nevertheless, averaged across the incubation, total biological O incorporation into released Pi was dominated by equilibrium fractionation (contribution of (*a* – *c*) to *a* in %, L = 99, M = 93, H = 94). Therefore, this study provided first direct evidence for the dominance of equilibrium fractionation during biological release of Pi in soil which was postulated but not yet disentangled in direct approaches for marine sediments (Blake et al. 2005, Goldhammer et al. 2011) and soils (Angert et al. 2011, 2012, Gross et al. 2015, Tamburini et al. 2012).

3.3.3 Reliability of underlying calculations and assumptions

A regression of $\delta^{18}O_{Pi/ti}$ values on $\delta^{18}O_{SW/ti}$ values of combinations of either two (two-point regression based on LM, LH, or MH) or three (three-point regression based on LMH) labeling treatments was used for the calculation of the total biological O incorporation factor *a* (**Figure 3.7**). The coefficient of variation (CV = standard deviation divided by the mean times 100) of three replicate analyses of one sample ranged between 16 % (LMH) and 77 % (MH) at the first day of incubation and between 4 % (LM) and 30 % (MH) after the second day of incubation onwards. Averaged across all incubation intervals, the regression based on three treatments was characterized by the lowest CV (LMH = 11 %, LH = 13 %, LM = 19 %, MH = 35 %). Therefore, the regression based on three isotopically labeled waters is the most robust and reliable one. However, a small labeling efficiency for the ^{18}O-depleted water (see **Section 3.3.1**) was encountered. Therefore, either the use of even more ^{18}O-depleted water or three ^{18}O-enriched waters (e. g. +50 ‰, +100 ‰, and +150 ‰) was recommended, the latter likely being more feasible as waters can be produced by diluting commercially available > 95 atom % $H_2{}^{18}O$. Despite some differences in the variation of *a* depending on the treatment combination used, the calculated total O incorporation factor *a* did not differ significantly among combinations (matched pairs t-test, $P > 0.25$). If three labeled waters were used to calculate the O incorporation factor *a*, the reproducibility of *a* for aliquots of one soil sample was associated with an uncertainty of around 10 % (CV [%]: 16, 7, 10, 11, and 10 at days one, two, four, seven, and 14, respectively). Remarkably, even if the shift in $\delta^{18}O_{Pi/ti}$ as compared to $\delta^{18}O_{Pi/t0}$ was small as was the case for day one (**Figure 3.6**), the reproducibility was still acceptable. Therefore, the calculation of the total O incorporation factor *a* as proposed here is a robust method yielding estimates with a reproducibility commonly observed if handling and analyzing soil samples (± 10 %).

The calculation of the kinetic O incorporation factor *c* was based on the assumption that monoesterase activity dominated (incorporation of one out of four O atoms from ambient water into the PO_4 molecule, see **(Equ. 2.5)**) and on mean published values for $\delta^{18}O_{Po}$ and $\varepsilon_{Pi/W}$ (Liang and Blake 2006b, 2009, Tamburini et al. 2014, von Sperber et al. 2014, 2015). It is generally assumed that monoesterase activity dominates over other phosphatase enzyme activities in soil (Margesin and Schinner 1994,

Nannipieri et al. 2011) though the relevance of mono- and diesterase activity might depend on soil conditions (Turner and Haygarth 2005). Even if it was assumed that two instead of one out four O atoms were incorporated from ambient water (0.5 instead of 0.25/0.75 in **(Equ. 2.5)**; (Liang and Blake 2009, Goldhammer et al. 2011, Tamburini et al. 2014)), the resulting *c* was identical up to the third decimal place (data not shown). At the moment there are no methods available to i) assess apparent fractionation factors associated with the release of Pi from Po/microorganisms nor to ii) separate Po from the mineral soil matric while guaranteeing the preservation of the original $\delta^{18}O_{Po}$ values so fixed values were used for $\varepsilon_{Pi/W}$ and $\delta^{18}O_{Po}$ published so far (Liang and Blake 2006b, McLaughlin et al. 2006, Paytan et al. 2002, von Sperber et al. 2014, 2015). Some authors analyzed $\delta^{18}O_{Po}$ values of pure compounds potentially contributing to Po in soil (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015, McLaughlin et al. 2006, Paytan et al. 2002). However, the methods used to convert Po to molybdate-reactive Pi that can be measured photometrically are not appropriate for hydrolyzing the complex mixture of Po compounds in soil. Both H_2O_2 addition followed by UV radiation (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015) or acid digestion at 50 °C low temperatures and for period of 24 to 36 h likely result in incomplete hydrolysis (McLaughlin et al. 2006) and thus, produce biased $\delta^{18}O_{Po}$ values for soil samples. In soil science, strong acids, high temperatures (sometimes combined with high pressure), and extended digestion periods are used to achieve complete Po hydrolysis (Kuo 1996). All of these conditions foster the abiotic exchange of O atoms from water of the digestion solution into hydrolyzed Pi (Blake et al. 1997, McLaughlin et al. 2006) and thus, again produce artifacts if measuring $\delta^{18}O_{Po}$ values in soil samples. Alternatively, operationally defined Po fractions in soil might be used that furthermore, carry information on bonding-strength/bioavailability of P (Hedley et al. 1982). Though some attempts have been published (Joshi et al. 2016, Zohar et al. 2010a, 2010b), the unambiguous separation of Po from Pi concomitantly extracted by the extraction solutions used (e. g. $NaHCO_3$ or NaOH) and the subsequent purification procedure to yield Ag_3PO_4 starting at alkaline conditions remains challenging. Once $\delta^{18}O_{Po}$ values can be determined reliably, this approach will offer the possibility of estimating the kinetic O incorporation factor *c* and the apparent isotopic fractionation factor $\varepsilon_{Pi/W}$ at the same time. Based on the experimental set up of this study, **(Equ. 3.2)** can be used for linear curve fitting resulting in an estimation of the unknown parameters *c* and $\varepsilon_{Pi/W}$. It was already tried this with three unknown parameters ($\delta^{18}O_{Po}$, *c*, and $\varepsilon_{Pi/W}$), but the number of data points in relation to the number of parameters to be estimated was too small to yield realistic results.

Nevertheless, to evaluate the influence of the selection of fixed values for $\delta^{18}O_{Po}$ and $\varepsilon_{Pi/W}$, a sensitivity analysis was conducted based on the results of day two and 14 (treatments M and H) representing small and large total O incorporation, respectively (**Supplementary material: Figure S 3** – Please note that no confidence intervals are displayed due to the high uncertainty associated with the probability

of a given $\delta^{18}O_{Po}$ or $\varepsilon_{Pi/W}$ value occurring in soil. This is because to the best of the knowledge neither of these two has ever been determined in soil samples.) The full range of $\delta^{18}O_{Po}$ and $\varepsilon_{Pi/W}$ that have been reported in the literature so far (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015) was used. In absolute terms, the kinetic O incorporation factor *c* differed by ≤ 0.01 (M and L at day two and 14) for the full range of $\delta^{18}O_{Po}$ values. Related to the total O incorporation factor *a*, this change equaled a change of the contribution of *c* to *a* (expressed as percentage) of ≤ 2 %. Depending on the isotopic fractionation factor $\varepsilon_{Pi/W}$, the kinetic O incorporation factor *c* differed by ≤ 0.01 (M and L at day two and 14). Again, related to the total O incorporation factor *a*, these changes modified the contribution of *c* to *a* by around 1 %. Therefore, regardless of the potentially great ranges ($\delta^{18}O_{Po}$ = 10 ‰, $\varepsilon_{Pi/W}$ = 50 ‰) in variables that were set fixed, the calculated kinetic O incorporation factor *c* and its contribution to the total O incorporation *a* hardly changed. Despite uncertainties in the true values for $\delta^{18}O_{Po}$ and $\varepsilon_{Pi/W}$, this approach provides robust and consistent results concerning the differentiation between kinetic and equilibrium fractionation contributing to biological Pi release in soil.

4 Method criticism

The presented data include some methodical errors that lead to uncertainty. The following chapter aims to provide a detailed discussion of these errors

As all chemical properties were measured at the same time on the same samples, there is no fluctuation with time and management like described by Buchanan and King (1992) (**Section 3.1, 3.2, 3.3**).

The organic layer was removed for all measurements. Therefore, Pmic was likely to be underestimated in forest soils. Zavišić et al. (2016) demonstrated, that Pi is 26 × higher and 6 × higher in the organic layer than in the mineral layer at low and high level P sites, respectively. However, if the organic layer in forests would have been included, a comparison between forests and grasslands would have been biased, as it would have included the contrast between the organic layer and the mineral soil (**Section 3.1**).

While the bulk of roots was removed by hand, an undefined amount of fine roots remained in the samples, which may cause overestimates of Pmic. Removal of roots also removes a large proportion of microorganisms present in the soil adhering to the root surface (McLaughlin and Alston 1987) (**Section 3.1, 3.2, 3.3**).

Pmic measurements were based on snapshot studies (i. e. results are based on a single round of sample collection (Burton et al. 2010)). Measurements by Regan et al. (2014) showed that microbial community spatial structure was positively correlated with the local environment, i. e. physical and chemical soil properties. The variability differs through the seasons. Especially, density and diversity of plants had additional effects in the summer period (Regan et al. 2014). Therefore, the measurements for this study were organized in May (spring time). The soil sampling campaign was performed within three weeks, because of the great quantity of sampling sites (3 study areas, each comprising 100 plots) (**Section 3.1, 3.2**).

For Pmic, the data used in this study were determined in 2011 and 2014. The dataset for P fractions ($NaHCO_3$-P, NaOH-P, HCl-P, H_2SO_4-P) were measured in 2008 and the vegetation records were specified in 2009. Therefore, these data might not exactly represent the situation of the studied years. Alt (2012) found that there are only low differences in P fractions especially labile Pi in soil. Correlations of $NaHCO_3$-Pi and resin-P (as part of Pmic) between different years show a strong correlation (**Table 4.1**) (**Section 3.1, 3.2**).

Table 4.1: Correlations of $NaHCO_3$-Pi and resin-P between different years (*r* = *Pearsons*'s rank correlation, *n* = sample size; *** *P* < 0.001).

	$NaHCO_3$-Pi (2008)	**$NaHCO_3$-Pi (2014)**	**resin-P (2011)**
$NaHCO_3$-Pi (2014)	*r* = 0.65*** *n* = 112		
resin-P (2011)	*r* = 0.50*** *n* = 113	*r* = 0.65*** *n* = 149	
resin-P (2014)	*r* = 0.44*** *n* = 113	*r* = 0.70*** *n* = 149	*r* = 0.77*** *n* = 150

The detection limit for photometrical P measurements with the continuous flow analyzer are dependent on the extraction solution and the measuring procedure, which means measurements of low- or high concentrations. The detection limit for the extract with 0.2 M HNO_3 was 0.04 ± 0.01 mg l^{-1} and 0.01± 0.00 mg l^{-1} and for the extract with 0.5 M $NaHCO_3$ 0.03 ± 0.01 mg l^{-1} and 0.01 ± 0.01 mg l^{-1} for high- and low concentration measurements, respectively. In interaction with a specific membrane system, for P measurements a linear relationship is guaranteed up to 7 mg l^{-1} (low) or 50 mg l^{-1} (high) for both HNO_3 and $NaHCO_3$ extract (**Section 3.1, 3.2, 3.3**).

There are some differences using conditioned resin stripes catching dissolved P. Within the photometer three digits for values are possible to measure. Taking three digits of P recovery is about 83.333 ± 3.333 % and 91.667 ± 1.054 % for low and high P spikes, respectively. Using two digits P recovery is about 100.00 ± 0.00 % independent of the P spike. Thus, resin-P might be underestimated. Furthermore, the company VWR International GmbH could not provide a safety data sheet for the used anion exchange membrane including the exchange capacity and recommended use. However, at least preliminary tests within the described methods showed that the exchange capacity was not reached (data not shown) (**Section 3.1, 3.2, 3.3**).

The observation of O incorporation is feasible, because the PO_4 radical is fairly inert to chemical redox reactions and to O exchange at low temperature (< 80 °C) (Blake et al. 2001). At low temperatures exchange of O atoms between the released Pi and the ambient water is only possible via biological activity (Blake et al. 2001) and it is independent of the isotopic composition of the added water (Longinelli 1965, Winter et al. 1940).Morevoer, there are no fractionations during precipitation (Liang and Blake 2007) and/or adsorption or desorption (Jaisi et al. 2010) of $^{16/18}O$ in PO_4. However, Blake et al. (2005) showed that microbes prefer to take up the lighter isotopes of PO_4 ($P^{16}O_4$) leading to an accumulation of heavier isotopes in the residual PO_4 ($P^{18}O_4$), the resin-P. As a matter of the proceeding time during the experiment and the unknown microbial pool, differences between the different treatments could be imagined. Intra-cellular PPase catalyses an exchange of all four O atoms of PO_4 and ambient water (Longinelli and Nuti 1973, Blake et al. 2005, Chang and Blake 2015). Hence, the $\delta^{18}O$ of resin-P ($\delta^{18}O_P$) of a given source (e. g. plant litter) might be equilibrated rapidly with $\delta^{18}O$ in soil

water ($\delta^{18}O_{SW}$), thereby masking the original isotopic source signal. After cell death and cell lysis, equilibrated Pi molecules are released into the environment. Thus, there is the chance that Pi released from dead cells dominates the O isotopic composition of Pi in environmental systems (Colman et al. 2005, Tamburini et al. 2012, von Sperber et al. 2014). Paytan et al. (2002) showed that O isotope composition of Pi can be rapidly altered by living organisms. Over time, the P inside the cells will be continuously processed and cycled, resulting in O isotope exchange and resetting the initial O isotope ratio (Paytan et al. 2002). Inorganic P released by extra-cellular phosphatase is different in O isotope compositions (Liang and Blake 2006b, 2009, von Sperber et al. 2014) in comparison to Pi released by intra-cellular PPase being not in equilibrium with cell water (Blake et al. 2005, Paytan et al. 2002) (**Section 3.3**).

The calculation of biological O incorporation factor (*a*) **(Equ. 2.4)** as slope of the regression was done for each incubated soil and incubation time separately. Generally, four combinations of labelling treatments are possible: 1) L + M + H, 2) L + M, 3) L + H and 4) M + H (**Figure 4.1**). The significance of calculated slope *a* was increasing with increasing entered replicates. In case of LMH the sample size (*n* = 9) entered in calculation is much higher as for LM, LH and MH (*n* = 6, respectively). Comparisons of each calculated *a* factor showed that there are no differences between combinations (**Figure 3.7**) (**Section 3.3**).

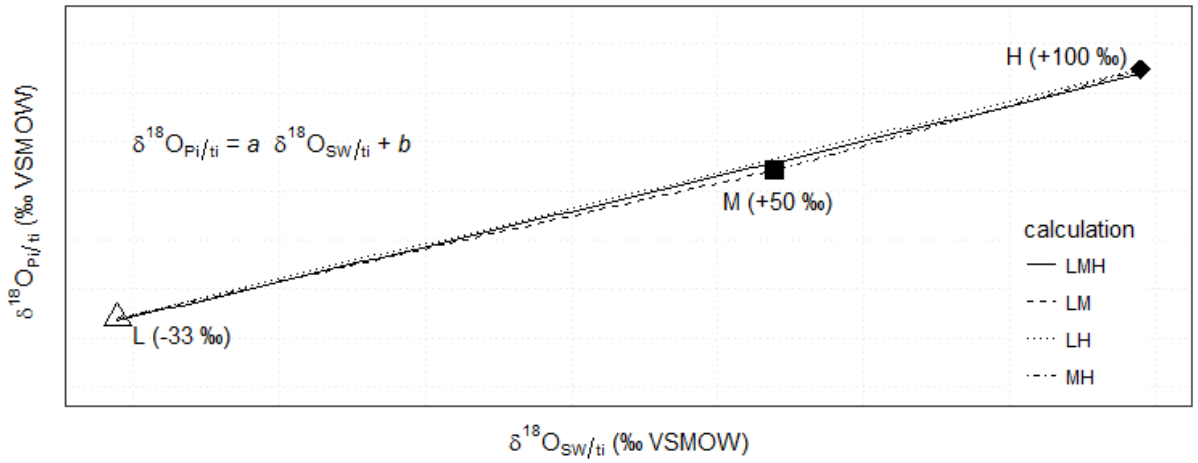

Figure 4.1: Calculation of biological O incorporation factor (*a*) calculated as the slope of the regression of $\delta^{18}O_{Pi/ti}$ on $\delta^{18}O_{SW/ti}$ values based on the combination of the labeling treatments (L = low [-33 ‰], M = medium [+50 ‰], H = high [+100 ‰]).

As at the moment no methods are available to i) assess apparent fractionation factors associated with the release of Pi from Po/microorganisms nor to ii) separate Po from the mineral soil matrix while guaranteeing the preservation of the original $\delta^{18}O_{Po}$ values (McLaughlin et al. 2006, Paytan et al. 2002)

fixed values were used for $\varepsilon_{Pi/W}$ and $\delta^{18}O_{Po}$ published so far (Liang and Blake 2006b, von Sperber et al. 2014, 2015) (**Section 3.3**).

As the labeled soils were incubated in air-tight preserving jars to minimize the evaporation of water and associated isotopic enrichment effects (Luz et al. 2009), the preservation of oxic conditions could not be assumed. Therefore, the monoesterase activity (AcidPase, APase) was observed as a metric for the redox-state of the jars. In general, there was no increasing or decreasing effect during the incubation time or labelling and no difference to background signal (**Figure 4.2**) (**Section 3.3**).

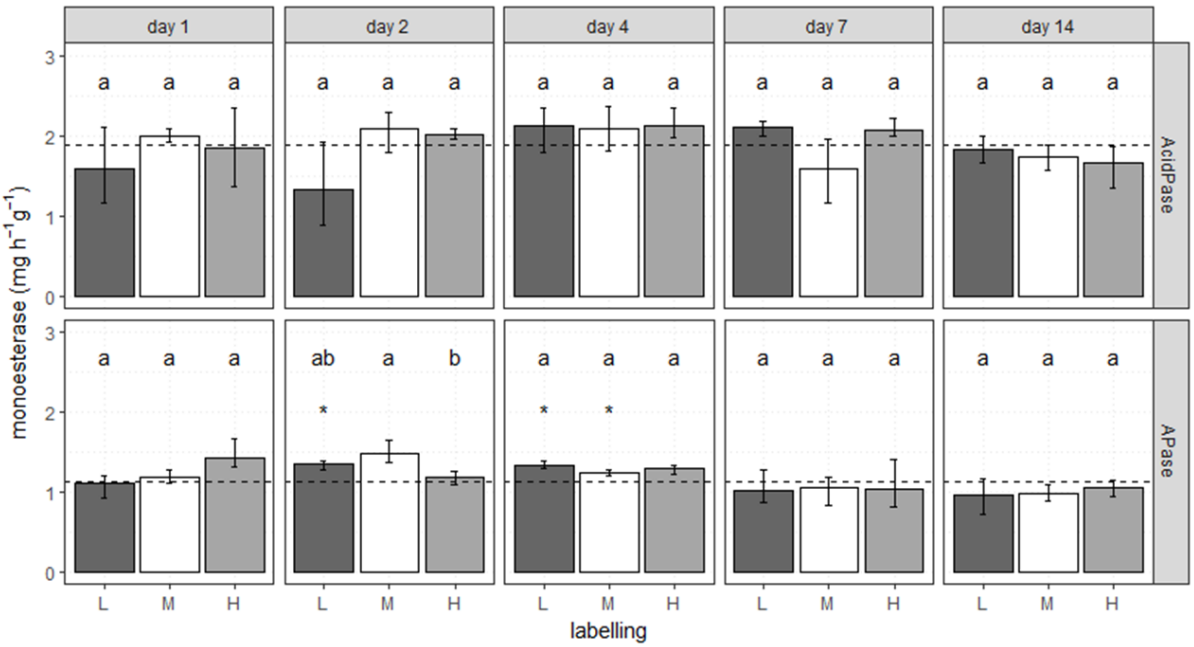

Figure 4.2: Mean concentrations of monoesterase (AcidPase and APase) in examined soil differentiated to day of incubation and labelling treatments (L = low [-33 ‰], M = medium [+50 ‰], H = high [+100 ‰]; error bars represent standard errors; different letters represent significant differences [$P < 0.05$]; dotted lines represent background signal, * indicates differences of mean to background signal)

The experimental setup for Ag_3PO_4 precipitation was done after Tamburini et al. (2010). Technically this procedure was performed for HCl-extracts. However, this study used 0.2 M HNO_3. For the method, besides removing organic matter, the conditions have to stay acidic, which is accounted for by using HNO_3 (personal communication Federica Tamburini, ETH Zürich, 03.06.2013). Nevertheless, a comparison of the various precipitation methods is needed. For precipitation of Ag_3PO_4 a minimal amount of 0.8 mg PO_4 is necessary (Weiner et al. 2011). Smaller samples show worse precision and a trend towards lower values (Colman 2002). Within this study the used amount of Ag_3PO_4 was > 0.8 mg P (mean = 1.2 mg P). Only two samples fell under the intended amount (0.6 mg P). One sample showed a tendency towards lower value in comparison to the repetitions. The reproducibility for $\delta^{18}O_{Pi}$ measurements is ± 0.3 ‰, taking into account that the yield of the standards was on average 97.8 %

with a standard deviation of 1.5 %. The measured samples (Ag_3PO_4) had a yield of 88.5 ± 1.1 % (**Section 3.3**).

5 Conclusions

Microbial P concentrations in soil differed among study regions. This effect may well be explained by different environmental conditions (pH, soil moisture, substrate availability for microorganism metabolism). In addition, Pmic concentrations were closely related to substrate availability (especially Corg) in both, forest and grassland soils. Analysis of PCA and regressions showed that abiotic and biotic variables controlling Pmic concentrations in soil are the same for forests and grasslands. Corg concentrations, soil moisture as well as soil pH, soil C : N ratios, and P fractions control Pmic concentrations in soil. Plant diversity was related indirectly to Pmic concentrations. There was no significant direct correlation if regions were analysed individually and plant diversity significantly explained a proportion of variance in Corg concentrations which itself was an important explanatory variable of Pmic concentrations. Regions with high annual precipitation, high Corg concentrations in soil, and high soil pH are characterized by the highest Pmic concentrations in soil (ALB > HAI, SCH). Analysing the three regions separately, neither tree species identity nor management (managed vs. near-natural) had significant effects on Pmic concentrations in forest soils. Also, in grassland soil, Pmic seems to be insensitive to management. Despite management effects on P fractions in soil and a relationship between selected P fractions and Pmic concentrations in soil, Pmic was found to be insensitive to forest and grassland management measures.

Microbial P concentrations in 2014 were lower than 2011. As well as 2011 Pmic concentration is soil differed among study regions (ALB > HAI > SCH). Lower Pmic concentrations in 2014 could be explained by higher precipitation and soil moisture resulting in a general loss of nutrients. In 2014 an effect of land use in management (meadow, mown pasture and pasture) in SCH was found and an effect of cutting in HAI. The negligible fertilization effect could be explained with lower P fertilization rates. Otherwise than Corg, Pmic was not influenced by soil type. Only Histosol stood out. So, the hypothesis that Pmic depending on soil type could be validate for Histosols only.

Within this study soil samples were incubated with waters differing in the O isotope composition and a linear regression of $\delta^{18}O_{Pi}$ values on $\delta^{18}O_{SW}$ values in soil water was used to quantify the total biological O incorporation into Pi during incubation. Using isotopically labeled water, this study was able to quantify the proportion of PO_4 molecules deriving from enzymatic hydrolysis of Po in soil precisely (CV $\leq$ 10%). Starting with a mean of 5 % (total biological O incorporation factor a = 0.05) one day after label addition, the proportion of molecules that had experienced biological O incorporation increased to 36 % in 14 days after label addition. During the incubation, this total biological O incorporation into released Pi was mainly due to enzymatic hydrolysis associated with equilibrium fractionation, i. e. the kinetic O incorporation factor c contributed less than 10 % to the total biological

O incorporation factor *a*. Further studies are necessary to test whether the predominance of enzymatic hydrolysis associated with equilibrium fractionation persists under a range of soil conditions (pH, composition of Po species, Pi availability, N vs. P limitation of plants and microorganisms). In particular, this approach could test if the deviation of $\delta^{18}O_{Pi/t0}$ values of a soil/sediment sample from expected $\delta^{18}O_{Pi/Equ}$ values (disequilibrium) can indeed be explained by processes associated with kinetic fractionation which was suspected to dominate in case of P-limiting conditions (Goldhammer et al. 2011). In conclusion, this approach offers unique insights into actual enzymatic processes involved in Pi release in soil which has not been possible so far based on standard enzyme assays.

For better management of P cycle in the ecosystem soil a better understanding Pmic as part of P cycle is mandatory. The incubation experiment showed that there is a possibility to disentangle between kinetic and equilibrium fractionation of PO_4 in natural (untreated) soil. In scientific continuation there should be measurements/calculations of Pmic within this method. With measurements of $\delta^{18}O_P$ the process of microbial turnover of P can be differentiated in more detail. It is well known that microbes prefer to take up lighter isotopes, but which isotope will be immobilized? the turnover of Pmic in soil *in situ* will be possible to follow. What will happen with the biochemical released PO_4? With the knowledge of influencing factors Pmic can be controlled in a better way. This understanding is necessary for a sustainable soil (P-)economy and could contribute to stable P cycle in the ecosystem, because P fertilizer, which is primarily rock derived, will be depleted in future.

Contributions

Some data were obtained from the BExIS after consultation with the owner (**Table 2.3**) (**Section 3.1, 3.2, 3.3**). Ulli Bange has done the laboratory work for Pmic 2011 (**section 3.1**). After the initial supervision of *Federica Tamburini* and *Emmanuel Frossard* at ETH Zürich I conducted the preparation of phosphate for $\delta^{18}O$ measurements at Tübingen on my own assisted by *Florian Schneider* and my student helpers. *Heiner Taubald* and *Bernd Steinhilber* measured $\delta^{18}O_{Pi}$ at Tübingen, *Lutz Breuer* and *Heike Weller* $\delta^{18}O_{SW}$ at Göttingen. *Ingo Schöning* provided the associated soil physical and chemical data (**Section 3.3**). Additional used data describing the study site are provided by *Ingo Schöning* (pH 2011, texture, Corg, total C, total N: **Section 3.1**, **3.2**, **3.3**), working group of *Ellen Kandeler* (pH 2014, enzyme activity, Cmic, Nmic: **Section 3.1**, **3.2**), working group of *Markus Fischer* and the managers of the three Exploratories (geographical characteristics, land-use information, biodiversity: **Section 3.1**, **3.2**, **3.3**). The owner of the data complemented methodical details on data generation (**section 2.5**, **2.6**) and commenting on first draft of **Section 3.1**. *Yvonne Oelmann* developed the scientific ideas building the basis for funding the project DYNPHOS within the Biodiversity Exploratories.

My own contributions to this thesis comprises planning of experiments, sample collection in the field, sample preparation and analyses in the laboratory, calculation and statistical analyses of the data, scientific interpretation of results, their discussion and the writing of the chapters and articles.

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Supplementary material

The role of chemical properties, land use and plant diversity for microbial phosphorus in forest and grassland soils

Table S 1: Relationship between soil moisture and soil texture in forest and grassland soils (*r* = *Pearsons*'s rank correlation, *n* = sample size; ns = not significant, * *P* < 0.05 ** *P* < 0.01, *** *P* < 0.001).

land-use type		forest soils		grassland soils	
variable		*r*	*n*	*r*	*n*
texture	clay	0.89***	150	ns	150
	fine silt	0.79***	150	ns	150
	medium silt	0.68***	150	0.38***	150
	coarse silt	0.47***	150	-0.24**	150
	fine sand	-0.73***	150	-0.25**	150
	medium sand	-0.86***	150	-0.22**	150
	coarse sand	-0.67***	150	ns	150

Table S 2: Output of each structural equation model on the relationships between different abiotic controlling factors, plant diversity and Pmic (ALB = Schwäbische Alb, HAI = Hainich-Dün, SCH = Schorfheide-Chorin).

Model	Model 1				Model 2			
	χ^2	df	probability level	RMSEA index	χ^2	df	probability level	RMSEA index
grassland soils								
all regions	1.98	1	0.159	0.081	7.91	1	0.005	0.217
ALB	0.01	1	0.975	< 0.001	0.78	1	0.379	< 0.001
HAI	0.86	1	0.353	< 0.001	2.59	1	0.108	0.180
SCH	0.01	1	0.931	< 0.001	0.04	1	0.834	< 0.001
forest soils								
all regions	19.81	1	< 0.001	0.355	23.71	1	< 0.001	0.390
ALB	10.71	1	0.001	0.445	11.13	1	0.001	0.455
HAI	5.77	1	0.016	0.312	11.00	1	0.001	0.452
SCH	0.01	1	0.932	< 0.001	0.03	1	0.853	< 0.001

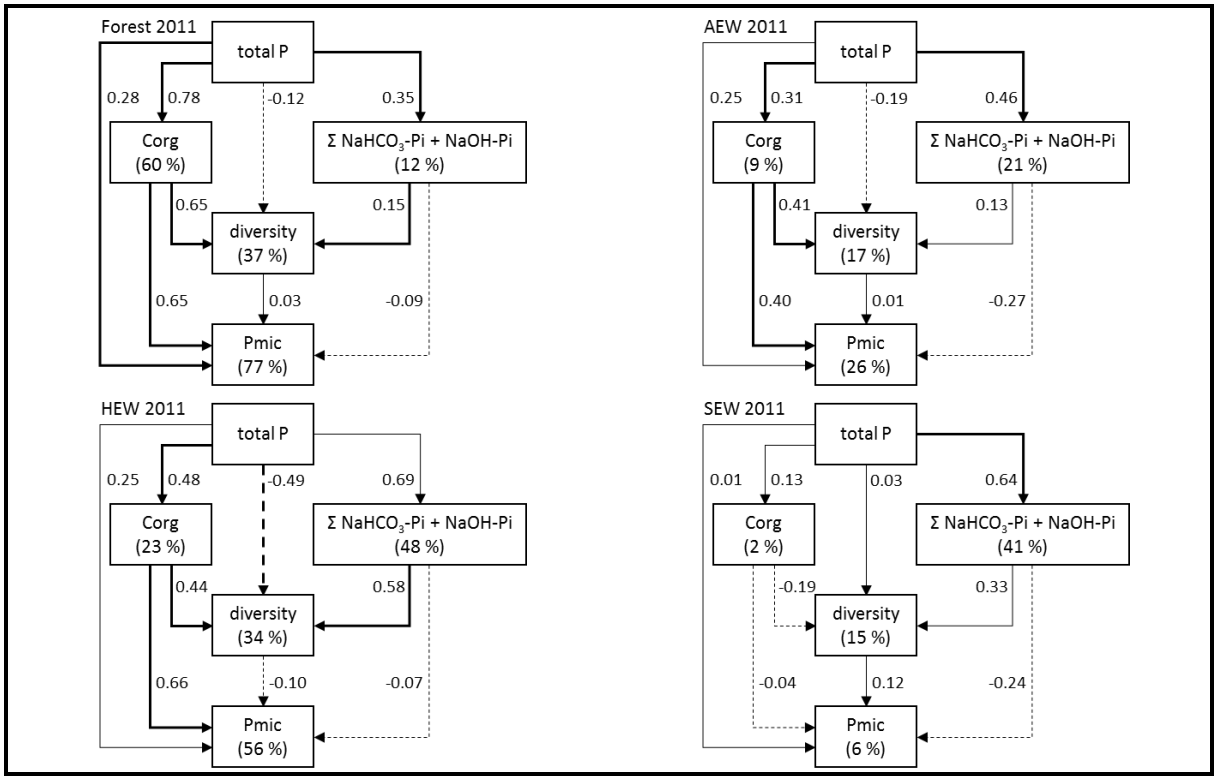

Figure S 1: Structural equation model 1 of the relationships between different abiotic controlling factors, plant biodiversity (diversity) and microbial phosphorus (Pmic) in forest sites for Forest (forest sites all three regions together, RMSEA = 0.355), AEW (forest sites Schwäbische Alb, RMSEA = 0.445), HEW (forest sites Hainich-Dün, RMSEA = 0.312) and SEW (forest sites Schorfheide-Chorin, RMSEA < 0.001). In this model, plant diversity is considered as response variable i.e., depending on organic carbon (Corg) concentrations. The percentage of explained variance of the variable is given in brackets. Numbers next to arrows represent standardized path coefficients. Path lines: bold lines = $P < 0.05$; thin lines = $P \geq 0.05$; solid line = positive path coefficient; dashed line = negative path coefficient.

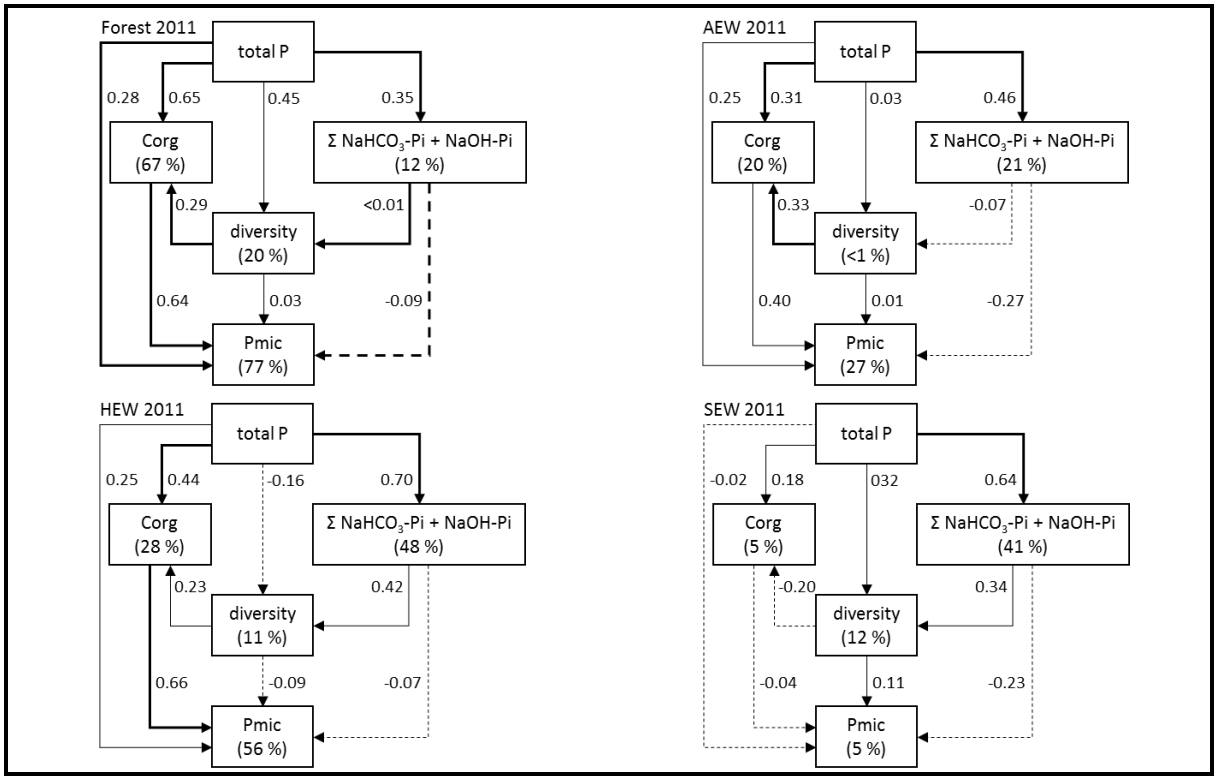

Figure S 2: Structural equation model 2 of the relationships between different abiotic controlling factors, plant biodiversity (diversity) and microbial phosphorus (Pmic) in forest sites for Forest (forest sites all three regions together, RMSEA = 0.390), AEW (forest sites Schwäbische Alb, RMSEA = 0.455), HEW (forest sites Hainich-Dün, RMSEA = 0.452) and SEW (forest sites Schorfheide-Chorin, RMSEA < 0.001). In this model, plant diversity is considered as independent variable i.e., controlling both organic carbon (Corg) and Pmic concentrations. The percentage of explained variance of the variable is given in brackets. Numbers next to arrows represent standardized path coefficients. Path lines: bold lines = $P < 0.05$; thin lines = $P \geq 0.05$; solid line = positive path coefficient; dashed line = negative path coefficient.

Microbial phosphorus of grassland sites – interannual variations

Table S 3: Climate data (standard error in brackets; na = not available; data: Wöllauer et al. (2015)).

region	April 2011	May 2011	April 2014	May 2014
Schwäbische Alb				
mean air temperature (°C)	10.7 [±0.2]	13.7 [±0.2]	9.8 [±0.2]	12.2 [±0.1]
precipitation (mm) *	na	na	63.8 [±5.2]	77.4 [±2.9]
mean soil moisture (%) **	39.8 [±1.0]	29.3 [±0.9]	32.7 [±1.3]	38.5 [±1.2]
mean soil temperature (°C)	10.2 [±0.2]	12.8 [±0.2]	9.7 [±0.2]	12.1 [±0.2]
Hainich-Dün				
mean air temperature (°C)	14.7 [±0.1]	14.7 [±0.1]	11.3 [±0.1]	13.2 [±0.1]
precipitation (mm) *	21.9 [±2.0]	27.2 [±3.5]	23.0 [±1.4]	115.5 [±8.9]
mean soil moisture (%) **	9.8 [±0.9]	20.9 [±0.7]	31.9 [±0.7]	32.7 [±0.7]
mean soil temperature (°C)	10.1 [±0.2]	13.6 [±0.2]	10.1 [±0.2]	12.8 [±0.2]
Schorfheide-Chorin				
mean air temperature (°C)	11.7 [±0.2]	14.3 [±0.2]	11.2 [±0.1]	13.0 [±0.1]
precipitation (mm) *	na	44.3	32.6 [±1.0]	63.2 [±3.8]
mean soil moisture (%) **	32.5 [±2.4]	25.8 [±2.4]	37.0 [±2.2]	32.6 [±2.2]
mean soil temperature (°C)	9.4 [±0.3]	13.0 [±0.3]	10.5 [±0.1]	12.7 [±0.1]

* mean per sampled plot; ** volumetric water content

Quantification of enzyme-mediated hydrolysis by means of the oxygen isotope ratio of phosphate in soils

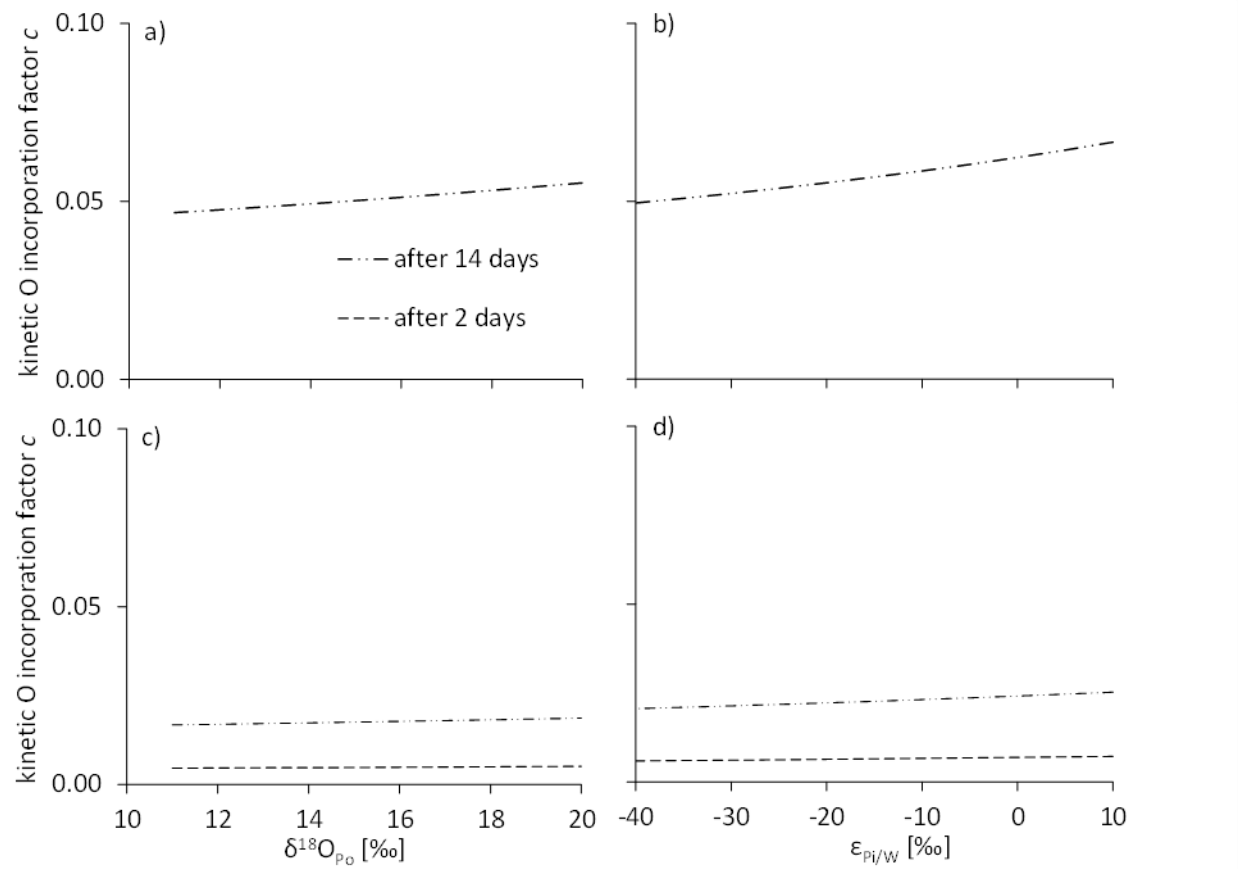

Figure S 3: Sensitivity analysis of the calculation of the kinetic O incorporation factor *c* using the range of $\delta^{18}O$ values of Po in soil ($\delta^{18}O_{Po}$) (a, c) and the isotopic fractionation factor $\varepsilon_{Pi/W}$ (b, d) published in the literature (Liang and Blake 2006b, 2009, von Sperber et al. 2014, 2015). The calculation was based on results of the M treatment (a, b; fixed variables: $\delta^{18}O_{Pi/t0}$ = 13.2, $\delta^{18}O_{Pi/t14}$ = 27.1, $\delta^{18}O_{SW/t14}$ = 33.8) and the H treatment (c, d; fixed variables: $\delta^{18}O_{Pi/t0}$ = 13.2, $\delta^{18}O_{Pi/t14}$ = 37.4, $\delta^{18}O_{SW/t14}$ = 58.9) with *a* = 0.12 and 0.36 at day 2 and 14, respectively. For the M treatment (a, b), the kinetic O incorporation factor *c* was zero across both ranges.

Table S 4: Selected geographical, physical and chemical characteristics of the sample site (AEG-06).

geographical	
longitude [a]	9°26’30’’
latitude [a]	48°24’4.5’’
soil type [b]	Leptosol
land use [c]	mown pasture
physical	
water content (%)	30.6
clay (g kg^{-1}) [d]	588
fine silt (g kg^{-1}) [d]	86
medium silt (g kg^{-1}) [d]	173
coarse silt (g kg^{-1}) [d]	121
fine sand (g kg^{-1}) [d]	27
medium sand (g kg^{-1}) [d]	5
coarse sand (g kg^{-1}) [d]	0
bulk density (g cm^{-3}) [d]	0.8
chemical	
pH [e]	5.9
inorganic Olsen-P (mg kg^{-1}) [f]	36.9
microbial P (mg kg^{-1}) [g]	65.0
Σ $NaHCO_3$-Po + NaOH-Po (mg kg^{-1}) [h]	400.2
total C (g kg^{-1}) [i]	78.1
inorganic C (g kg^{-1}) [i]	0.4
organic C (g kg^{-1}) [i]	77.7
total N (g kg^{-1}) [i]	7.8
C : N ratio [i]	10.0

[a] Nieschulze and Schulze (2016), [b] Fischer et al. (2010), [c] Hailer and Ayasse (2016), [d] Schöning et al. (2011), [e] Schöning and Trumbore (2015), [f] Sorkau and Oelmann (2015b), [g] Sorkau and Oelmann (2016), [h] Alt and Oelmann (2010), [i] Schöning et al. (2015)

Table S 5: Compilation of all results of this study.

treatment	replicate	ti (day)	resin-extractable Pi (mg kg^{-1})	$\delta^{18}O_{Pi}$ (‰)	$\delta^{18}O_{SW}$ (‰)	AcidPase (mg *p*-NP h^{-1} g^{-1})	APase (mg *p*-NP h^{-1} g^{-1})
back-ground	I	0	23.4	12.6	-10.3	1.8	1.0
	II	0	15.1	4.5	-10.1	2.0	1.1
	III	0	14.9	13.8	-10.3	1.8	1.3
L (-33 ‰)	I	1	22.2	14.0	-10.9	2.1	1.2
	II	1	24.0	14.1	-10.9	1.5	1.2
	III	1	12.4	12.3	-10.9	1.2	0.9
	I	2	14.9	12.2	-10.7	0.9	1.4
	II	2	12.4	12.9	-9.6	1.9	1.3
	III	2	15.5	14.0	-11.0	1.2	1.4
	I	4	15.6	12.9	-10.9	1.8	1.3
	II	4	22.2	12.9	-11.1	2.2	1.3
	III	4	22.0	13.5	-11.2	2.3	1.4
	I	7	15.1	12.5	-9.5	2.0	0.9
	II	7	17.8	13.6	-11.3	2.2	1.3
	III	7	17.8	13.5	-10.8	2.2	0.9
	I	14	13.7	8.9	-10.8	1.7	1.0
	II	14	15.1	13.7	-10.9	2.0	0.7
	III	14	11.8	13.4	-11.4		1.2
M (+50 ‰)	I	1	8.2	13.4	42.3	1.9	1.1
	II	1	23.2	18.0	36.2		1.3
	III	1	13.7	13.8	40.2	2.1	1.1
	I	2	18.4	18.7	38.1	2.3	1.6
	II	2	21.8	19.6	34.3	2.2	1.4
	III	2	18.4	19.1	34.8	1.8	1.4
	I	4	17.4	22.6	36.1	2.4	1.3
	II	4	17.4	22.1	36.7	1.8	1.2
	III	4	18.0	21.7	37.5	2.1	1.2
	I	7	13.0	25.8	36.0	2.0	0.8
	II	7	10.0	24.5	37.4	1.6	1.2
	III	7	13.9	25.1	34.2	1.2	1.2
	I	14	11.8	25.4	35.0	1.7	1.1
	II	14	14.3	28.5	33.7	1.6	
	III	14	12.5	27.3	32.9	1.9	0.9
H (+100 ‰)	I	1	24.4	19.7	58.9	1.8	1.7
	II	1	17.2	18.2	62.9	2.4	1.3
	III	1	11.7	14.8	56.2	1.4	1.3
	I	2	17.1	20.1	61.6	2.1	1.3
	II	2	15.9	22.3	58.7	1.9	1.2
	III	2	17.6	22.2	61.7		1.1
	I	4	18.1	26.0	58.8	2.3	1.3
	II	4	20.0	26.7	56.0	2.0	1.3
	III	4	12.8	23.3	58.5	2.0	1.2
	I	7	17.9	34.6	55.8	2.2	1.4
	II	7	13.6	32.6	57.3	2.0	0.9
	III	7	8.8	29.1	59.8	2.0	0.8
	I	14	13.4	38.6	56.8	1.8	0.9
	II	14	13.7	36.2	60.2	1.4	1.2
	III	14	15.0	37.3	59.8	1.9	1.1

Eigenständigkeitserklärung

„Ich erkläre hiermit, dass ich die zur Promotion eingereichte Arbeit mit dem Titel: „Land use and biodiversity effects on P-transformation in soil“ selbständig verfasst, nur die angegebenen Quellen und Hilfsmittel benutzt und wörtlich oder inhaltlich übernommene Stellen (alternativ: Zitate) als solche gekennzeichnet habe. Ich erkläre, dass die Richtlinien zur Sicherung guter wissenschaftlicher Praxis der Universität Tübingen (Beschluss des Senats vom 25.05.2000) beachtet wurden. Ich versichere an Eides statt, dass diese Angaben wahr sind und dass ich nichts verschwiegen habe. Mir ist bekannt, dass die falsche Abgabe einer Versicherung an Eides statt mit Freiheitsstrafe bis zu drei Jahren oder mit Geldstrafe bestraft wird.“