Microbial phosphorus in soils influenced by chemical properties, land use, and biodiversity

Corresponding author:
Yvonne Oelmann
Geoecology, University of Tübingen, Rümelinstr. 19-23, 72070 Tübingen, Germany
Email: yvonne.oelmann@uni-tuebingen.de
phone: + 49 7071 29 72398
fax: +49 7071 29 5378

This document is the accepted manuscript version of the following article:
Title

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

Authors

Elisabeth Sorkau¹, Steffen Boch², Runa S. Boeddinghaus³, Michael Bonkowski⁴, Markus Fischer⁵, Ellen Kandeler⁶, Valentin H. Klaus⁶, Till Kleinebecker⁶, Sven Marhan³, Jörg Müller⁷, Daniel Prati⁵, Ingo Schöning⁸, Marion Schrumpf⁸, Jan Weinert⁴, Yvonne Oelmann¹*

¹Geocology, University of Tübingen, Rümelinstr. 19-23, 72070 Tübingen, Germany
²Department of Biodiversity and Conservation Biology, Ecosystem Dynamics, Swiss Federal Research Institute WSL, Zürcherstrasse 111, CH-8903 Birmensdorf
³Institute of Soil Science and Land Evaluation, Department of Soil Biology, University of Hohenheim, Emil-Wolff-Str. 27, 70593 Stuttgart, Germany
⁴Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Str. 47b, 50674 Köln, Germany
⁵Institut of Plant Sciences and Botanical Garden, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland
⁶Institute of Landscape Ecology, University of Münster, Heisenbergstr. 2, 48149 Münster, Germany
⁷Institute for Biochemistry and Biology, University of Potsdam, Maulbeerallee 1, 14469 Potsdam, Germany
⁸Department for Biogeochemical Processes, Max-Planck-Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany

Keywords (4 – 6)
age class forest, unmanaged forest, land-use intensity, meadow, pasture, microbes
Abstract

Management intensity modifies soil properties, e.g., organic carbon (C_{org}) concentrations and soil pH with potential feedbacks on plant diversity. These changes might influence microbial P concentrations (P_{mic}) in soil representing an important component of the P cycle. Our objectives were to elucidate whether abiotic and biotic variables controlling P_{mic} concentrations in soil are the same for forests and grasslands, and to assess the effect of region and management on P_{mic} concentrations in forest and grassland soils as mediated by the controlling variables. In three regions of Germany, Schwäbische Alb, Hanich-Dün, and Schorfheide-Chorin, we studied forest and grassland plots (each n = 150) differing in plant diversity and land-use intensity. In contrast to controls of microbial biomass carbon (C_{mic}), P_{mic} was strongly influenced by soil pH, which in turn affected phosphorus (P) availability and thus microbial P uptake in forest and grassland soils. Furthermore, P_{mic} concentrations in forest and grassland soils increased with increasing plant diversity. Using structural equation models, we could show that soil C_{org} is the profound driver of plant diversity effects on P_{mic} in grasslands. For both forest and grassland, we found regional differences in P_{mic} attributable to differing environmental conditions (pH, soil moisture). Forest management and tree species showed no effect on P_{mic} due to a lack of effects on controlling variables (e.g., C_{org}). We also did not find management effects in grassland soils 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. We conclude that variables controlling P_{mic} or C_{mic} in soil differ in part and that regional differences in controlling variables are more important for P_{mic} in soil than those induced by management.
1 Introduction

In recent years, it has become apparent that phosphorus (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 nitrogen (N) inputs due to combustion of fossil fuels and N-based fertilizers may further shift terrestrial systems towards P-limitation (Holland et al., 2005).

The importance of soil microbial biomass P ($P_{\text{mic}}$) in the P cycle was emphasized by several studies (Chen et al., 2000; Oberson and Joner, 2005; Richardson and Simpson, 2011; Turner et al., 2013). However, the understanding of environmental conditions as drivers of $P_{\text{mic}}$ in soil is still in its fledgling stage. Only a few variables suspected to control $P_{\text{mic}}$ in soil have been included in the studies published so far and these did not consider the influence of plants. First, organic carbon ($C_{\text{org}}$) concentrations correlated with $P_{\text{mic}}$ concentrations in forests and grasslands (Joergensen et al., 1995; Stutter et al., 2015). This is probably related to the role of $C_{\text{org}}$ as ultimate energy source of heterotrophic soil organisms constraining the growth of microorganisms (Wardle, 1992). Second, Liebisch et al. (2014) found increasing $P_{\text{mic}}$ concentrations with increasing soil moisture in a grassland fertilization trial which is in line with a positive effect of soil moisture on microbial biomass carbon ($C_{\text{mic}}$) concentrations in soil in a meta-analysis (Wardle, 1998). Third, Liebisch et al. (2014) found highest $P_{\text{mic}}$ concentrations in soils with high pH (6.8). This effect might derive from the strong pH control of $H_2PO_4^-/HPO_4^{2-}$ ion concentrations in soil solution serving as the pool of P readily available for uptake by soil microorganisms. Fourth, total P concentrations in soil have been shown to be decisive for $P_{\text{mic}}$ concentrations in forest soils under beech (Joergensen et al., 1995). In addition, as microbes form 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). This is because microbes can act as a source and sink of P while being involved in transformation reactions of organic P ($P_o$) and inorganic P ($P_i$) fractions in soil (Tate, 1984; Brookes, 2001; Griffiths et al., 2012; Burns et al., 2013). Finally, plants might influence some of these controlling variables thereby also exerting an effect on $P_{\text{mic}}$ in soil. In artificial
grasslands and established forests, it has been shown that increasing plant diversity leads to increased $C_{\text{org}}$ stocks likely due to increased above- and belowground litter input (Fornara and Tilman, 2008; Steinbeiss et al., 2008; Gamfeldt et al., 2013; Lange et al., 2015). Whereas previous studies focused on a smaller set of land-use types and environmental factors driving $P_{\text{mic}}$ storage in microorganisms, we are the first to use a larger data basis is required for generalizing these results and potentially uncover environmental conditions controlling $P_{\text{mic}}$ in soil that have not yet been accounted for in the above-mentioned studies (C : N ratios and P fractions in soil). Recently developed statistical tools such as structural equation models (SEM) allow to separate direct from indirect effects of such large data sets based on linear regressions (Grace et al., 2012).

If $P_{\text{mic}}$ concentrations are studied under different climatic conditions, e. g. precipitation feeding back on soil moisture and other controls (parent rock material, soil pH, P fractions in soil, $C_{\text{org}}$, and plant diversity) we hypothesize differences in $P_{\text{mic}}$ among regions. Regional differences might be further reinforced by land use and the associated management. In forests, one component of management is associated with the selected tree species or mixtures and accordingly different harvest intervals. Tree species influence soil properties such as pH and C : N ratios thereby potentially affecting $P_{\text{mic}}$ concentrations in soil. Because tree species might well have been planted according to tree species performance, e. g. coniferous trees at sites with low soil pH, cause and effect are difficult to disentangle in established, non-natural ecosystems. Nevertheless, the above-mentioned correlations remain. To the best of our knowledge, only one study has been published so far on tree species effects on $P_{\text{mic}}$ concentrations in soil. In this study, two Mediterranean oak species resulted in different $P_{\text{mic}}$ Concentrations in soil (Aponte et al., 2013).

In grasslands, management affects $P_{\text{mic}}$ concentrations in soil via fertilizer inputs potentially associated with indirect effects, such as changes in soil pH and subsequent availability of P. In agriculture, the most common P containing fertilizers are inorganic fertilizers (produced from rock phosphates), manure or composts (Ayaga et al., 2006) which increase substrate availability for microorganisms. Another management option in grasslands is grazing. Reduced grazing (and
fertilization) intensity increased microbially-mediated processes resulting in microbial immobilization of scarce nutrients including P (Stutter et al., 2015). Therefore, increased $P_{mic}$ concentrations can be expected for low management intensity. But the opposite might also be true, i.e. high management intensity in terms of grazing and fertilization associated with high $P_{mic}$ concentrations in soil. This might be caused by positive effects of fertilization (as reasoned above) combined with the ability of microorganisms to accumulate P as polyphosphates in situations of “luxury P supply” (Condron et al., 2005).

The main objectives of our study were (1) to elucidate whether abiotic and biotic variables controlling $P_{mic}$ concentrations in soil are the same for forests and grasslands, and (2) to assess the effect of region and management on $P_{mic}$ concentrations in forest and grassland soils as mediated by the controlling variables.

We hypothesized that
i) $C_{org}$ concentrations, soil moisture as well as soil pH, soil C : N ratios, and P fractions control $P_{mic}$ concentrations in soil with plant diversity exerting an additional effect in forests and grasslands;

ii) regions with high annual precipitation, high $C_{org}$ concentrations in soil, and high soil pH will be characterized by highest $P_{mic}$ concentrations in soil (ALB > HAI, SCH);

iii) in forests, $P_{mic}$ 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, $P_{mic}$ concentrations increase with increasing land-use intensity due to fertilization.

2 Material and methods

2.1 Study regions

Our 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 assessed (Fischer et al., 2010). The project includes plots in three study regions in Germany differing in land-use intensity and biodiversity: Schwäbische Alb (ALB), Hainich-Dün (HAI), and Schorfheide-
Chorin (SCH). Each region (Table 1) comprises of 50 plots in grassland and 50 plots in forests resulting in a total number of 300 plots. Plots in each region have been used 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).

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, in which single or small groups of trees were harvested selectively. The forest in the ALB and HAI are dominated by European beech \( [Fagus sylvatica \text{ L.} ] \) (ALB: \( n = 38 \); HAI: \( n = 46 \)) and Norway spruce \( [Picea abies (L.) \text{ Karst}] \) (ALB: \( n = 12 \); HAI: \( n = 4 \)). In the studied forests in SCH European beech \( (n = 21) \), sessile oak \( [Quercus petraea \text{ Liebl.}] \) and/or English oak \( [Quercus robur \text{ L.}] \) \( (n = 7) \), and Scots pine \( [Pinus sylvestris \text{ L.}] \) \( (n = 22) \) are the dominating tree species.

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 one 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, \( n = 61 \)).

The fertilization of P comprises application of organic fertilizers (e. g. liquid manure) and mineral-fertilizer application. Average fertilizer application rates to fertilized grasslands during the last vegetation period were \( 11 \pm 2.6 \) kg P ha\(^{-1} \), \( 4 \pm 1.2 \) kg P ha\(^{-1} \), and \( 5 \pm 2.2 \) kg P ha\(^{-1} \) in ALB, HAI and SCH, respectively (Alt et al., 2011). In addition to fertilization differences in mowing frequency (number of cuts per year) and grazing intensity (livestock units \( \times \) grazing days ha\(^{-1} \) year\(^{-1} \)) were included.
2.3 Soil sampling

Soil samples (grassland: n = 150; forest: n = 150) were taken with soil corers (50 mm Ø, Eijelkamp, Giesenbeck, The Netherlands) in May 2011. 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). This time frame also reduced potential seasonal effects on Pmic (Regan et al., 2014) given that samples were collected in three regions. For each plot we used 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. As a standard procedure for the coordinated soil sampling campaign, organic layers were removed prior to mineral soil sampling at forest sites. We are aware that this procedure results in an underestimation of Pmic concentrations, but it facilitates the comparison between grasslands and forests as we relied on mineral soil samples only. Soil samples were sieved (< 2 mm) to remove stones and roots to minimize the influence of living plant tissue (McLaughlin and Alston, 1987). To avoid quantitative and qualitative changes in microbial biomass we used fresh soil samples for soil biological analyses (stored at 4 °C). Further soil chemical analyses were performed on air-dried soil samples.

2.4 Soil analyses

For analysing soil water content an aliquot of soil was dried to a constant weight at 105°C arriving at a “dry” sample weight. The weight of the moist and dry soil was recorded and the percentage water per dry soil calculated (Gardner, 1986). To determine Pmic we used a combination of methods by Kouno et al. (1995) and McLaughlin et al. (1986). The underlying principle is to lyse microbial cells of a field-fresh soil sample by 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 has to 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, we used resin stripes (anion exchange membranes, product code: 551642S VWR, Bruchsal, Germany) to extract P from 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$_2$O$_{dest}$) was added to each tube. The first aliquot was fumigated with 1 ml liquid hexanol (fumigated aliquot). Only H$_2$O$_{dest}$ was added to the second aliquot (non-fumigated aliquot) whereas in addition 1 ml of 20 µg P ml$^{-1}$ (as dissolved KH$_2$PO$_4$) was added to the third aliquot (P spike). The samples were horizontally shaken for 16 hours with bicarbonate resin membrane strips. Afterwards, the resin stripes were removed from the soils suspension, rinsed with H$_2$O$_{dest}$ to remove adhering soil, and transferred to new tubes. After that, 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 (P$_{hex}$), we used a sorption curve between non-fumigated and P spiked aliquots (Bünemann et al., 2008). Phosphorus concentrations in the fumigated sample are a function of microbial P (P$_{hex}$) 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. 1).

$$P (fumigated\ \text{aliquot}) = a \cdot \text{microbial} \ P (P_{hex}) + b \quad \text{(Equ. 1)}$$

Equation 1 can be rearranged to solve for microbial P (Equ. 2):

$$\text{microbial} \ P (P_{hex}) = \frac{P (fumigated\ \text{aliquot}) - b}{a} \quad \text{(Equ. 2)}$$

The slope $a$ is calculated as follows (Equ. 3):

$$a = \frac{P (P \text{ spike}) - P (non-fumigated\ \text{aliquot})}{P_{mass}(P \text{ spike})} \quad \text{(Equ. 3)}$$
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) we did not use a transformation factor for the calculated \( P_{\text{hex}} \) concentrations and used \( P_{\text{mic}} \) synonymously for \( P_{\text{hex}} \). We used hexanol instead of chloroform, because it is just as effective as chloroform with less hazardous nature (McLaughlin et al., 1986). Noteworthy, Bergkemper et al. (2016) reported lower \( P_{\text{mic}} \) 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 \( ^{33}\text{P} \) in resin-P were not 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 \( P_{\text{mic}} \) by hexanol fumigation of soils.

Soil microbial biomass carbon (\( C_{\text{mic}} \)) and nitrogen (\( N_{\text{mic}} \)) were determined using the chloroform fumigation extraction method (Vance et al., 1987). In brief, 10 g soil subsamples were fumigated with chloroform for 24 h and then extracted with 40 ml 0.5 M \( \text{K}_2\text{SO}_4 \) 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.

Phosphorus occurs in soils in various forms: \( \text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-} \) ions in soil solution and adsorbed to mineral surfaces (labile: \( \text{NaHCO}_3-P \)), P more strongly sorbed to iron and aluminium oxides (moderately labile: \( \text{NaOH}-P \)), P bound in calcium phosphates (stable: \( \text{HCl}-P \)) and occluded \( \text{P} \), e.g. spatially separated from \( \text{P} \) transformation reactions in secondary minerals such as pedogenic oxides (stable: \( \text{H}_2\text{SO}_4-P \)) (Cross and Schlesinger, 1995; Hedley et al., 1982; Negassa and Leinweber, 2009). For \( \text{P} \) fractions in soil, we used data published by Alt et al. (2011). Because of inaccessibility of some sites (e.g. because of extraordinarily high soil moisture), for \( \text{P} \) 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 \( \text{P} \) 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$_2$SO$_4$-P). First, 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. Second, 30 ml 0.1 M NaOH was added to the remaining soil followed by shaking for 16 h, decantation and filtration. Third, 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. Fourth, the residual soil mass was stored overnight in a porcelain crucible in a muffle furnace at 550 °C to destroy all organic material. Thereafter, 20 ml 0.5 M H$_2$SO$_4$ 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 P$_i$ 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 $\lambda$ = 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, $\lambda$ = 213.617 nm). For the labile and moderately fractions (NaHCO$_3$-P, NaOH-P), P$_o$ was calculated by subtracting P$_i$ from total dissolved P concentrations in the extracts. In addition to the individual fractions, inorganic P in the labile and moderately labile fraction was calculated as the sum of NaHCO$_3$-P$_i$ and NaOH-P$_i$ ($\Sigma$ NaHCO$_3$-P$_i$ + NaOH-P$_i$) and organic P in the labile and moderately labile fraction as the sum of NaHCO$_3$-P$_o$ and NaOH-P$_o$ ($\Sigma$ NaHCO$_3$-P$_o$ + NaOH-P$_o$). 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. 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 C$_{org}$ at
a temperature of 450 °C for 16 h (Solly et al., 2014). Then \( C_{\text{org}} \) was calculated as the difference between total and inorganic C.

### 2.5 Vegetation

Plant diversity was measured as number of vascular plant species. In 2009, we sampled vascular plants in all plots. In grasslands, we conducted this survey once between mid-May and end-of-June in an area of 4 m × 4 m (Socher et al., 2012), when the vegetation was developed best and the meadows were not mown yet. In forests, the vegetation changes strongly during the seasons with high abundances of early flowering geophytes in spring to high abundances of later flowering species in summer. To account for these differences and sample the total plot diversity, we recorded the vegetation of 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. To assess the total plant diversity per plot, we then combined the spring and summer records (Boch et al., 2013).

### 2.6 Statistical analyses

Results within the text are shown as mean [± standard deviation] if not specified otherwise. To test for significant differences in mean \( P_{\text{mic}} \) concentrations among regions and land-use types, we used an analysis of variance with Tukey Post Hoc tests. Correlations among variables were studied using Pearson’s rank correlations. To disentangle complex interactions among variables controlling \( P_{\text{mic}} \) in grassland and forest soils and to overcome limitations associated with bivariate correlations we used structural equation models (Grace et al., 2012). We structured different models to check for the direct and indirect effect on \( P_{\text{mic}} \) and disentangle between driving factor \( C_{\text{org}} \) (Model 1: \( C_{\text{org}} \) influencing \( P_{\text{mic}} \) and plant diversity) and plant diversity (Model 2: plant diversity influencing \( P_{\text{mic}} \) and \( C_{\text{org}} \)), respectively. As evaluation criterion for model quality we used probability level and root mean square error of approximation (RMSEA). To reduce the number of abiotic variables for the SEM we used a principal component analysis (PCA) with varimax rotation. We checked whether the
prerequisites for a PCA were met by Bartlett’s Test of Sphericity (Bartlett $P$) and the Kaiser-Meyer-Olkin index (KMO) (Abdi and Williams, 2010). We set the threshold for meaningful components to $\text{Eigenvalues} > 1$. Thereafter, we identified those variables 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. For $P_{\text{mic}}$ concentrations, we had to remove some outliers calculated after Grubbs and Beck (1972) from the data set (number of outliers: ALB: grassland = 2, forest = 2; HAI: grassland = 4, forest = 3; SCH: grassland = 4, forest = 12).

3 Results

3.1 Forests

Across all forest soils, $P_{\text{mic}}$ concentrations increased with increasing concentrations of $\text{NaOH-P}_o$, $\Sigma \text{NaHCO}_3-P_i + \text{NaOH-P}_o$, $\text{HCl-P}$, $\text{H}_2\text{SO}_4-P$, total $P$, total $N$, $C_{\text{org}}$, $C_{\text{mic}}$, $N_{\text{mic}}$ concentrations, as well as $\text{pH}$ and plant diversity, and decreased with increasing $\text{NaHCO}_3-P_i$, $\text{NaHCO}_3-P_o$ concentrations, $C:N$ ratio and $C_{\text{mic}}:N_{\text{mic}}$ ratio (Table 2). Microbial $P$ concentrations correlated significantly positively with soil moisture and clay contents (Table 2). At the same time, soil moisture was higher in fine-textured soils (Table S1). The ratios of $C_{\text{mic}}:P_{\text{mic}}$ ranged from 5 to 213 with an average of 22 (median = 16). With increasing plant diversity, $\text{NaHCO}_3-P_i$ concentrations and $C_{\text{mic}}:P_{\text{mic}}$ ratios decreased ($r = -0.19$ and $-0.27$, respectively; $P < 0.05$). The three regions differed in their overall $P_{\text{mic}}$ concentrations in the order ALB > HAI > SCH (Figure 1a). Separated according to region, neither forest management influenced $P_{\text{mic}}$ concentrations significantly nor did tree species ($P > 0.05$; Table 3). 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, we selected the following variables: total $N$ concentrations (Component 1; loading 0.91), $\text{NaHCO}_3-P_i$ concentrations (Component 2; loading 0.90), $\text{NaOH-P}_i$ (Component 3; loading 0.94). If data sets of grasslands and forests were merged, the PCA...
identified two further influential variables, i.e. C\text{org} concentrations (loading 0.91) and soil pH (loading 0.85). Because of similar loadings (and a close bivariate correlation between total N and C\text{org} concentrations; \( r = 0.98, \ P < 0.001 \)) and the desired consistency for grassland and forest SEMs, we replaced total N concentrations by C\text{org} concentrations in the final model. We used two SEMs treating plant diversity either as a response variable (depending among others on C\text{org} concentrations, Model 1; Figure S1) or as a variable controlling C\text{org} concentrations (Figure S2). For forest soils, SEMs could not be adequately fitted to P\text{mic} concentrations as probability levels < 0.02 and RMSEA > 0.3 indicated major deviations between data and models if regions were analysed separately (Supplementary Table S2, Figure S1, Figure S2). The one exception was the SCH characterized by a better fit between data and model, which however did not include paths significantly explaining P\text{mic} concentrations. If all regions were analysed together (ALL), the deviation between data and model was smaller (Supplementary Table S2). In both models, paths including plant diversity were significant.

3.2 Grasslands

Overall, P\text{mic} increased with increasing pH, concentrations of NaOH-P\text{org}, total P, \( \Sigma \text{NaHCO}_3\cdot\text{P}_\text{org} + \text{NaOH} \cdot\text{P}_\text{org} \), N\text{mic}, total N, C\text{mic}, C\text{org}, and plant diversity and decreased with increasing NaHCO\text{3-P}i concentrations (Table 2). P\text{mic} concentrations were related to soil moisture and clay contents (Table 2) while soil moisture was higher in fine-textured soils (Table S1). In grassland soils, we measured a mean C\text{mic} : P\text{mic} ratio of 14 (median = 12) with a range of 2 to 118. With increasing plant diversity, NaHCO\text{3-P}i and C\text{mic} : P\text{mic} ratio decreased (\( r = -0.62 \) and \( -0.24 \), respectively; \( P < 0.01 \)).

Mean concentrations of P\text{mic} in grassland soils of the three different regions followed the same order as in forest soils: ALB > HAI ≥ SCH (Figure 1b). 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 Chapter 2.2) and there were also no significant differences in P\text{mic} 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). In addition, we found no influence of cutting frequency on $P_{\text{mic}}$ concentrations (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). 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, we selected the following variables: NaOH-$P_i$ (Component 1; loading 0.81), HCl-$P$ (Component 2; loading 0.90), NaHCO$_3$-$P_i$ concentrations (Component 3; loading -0.81). We replaced HCl-$P$ concentrations by the variable with the second-highest loading in Component 2, i. e. total P concentrations (loading 0.84). The SEM thus included the same variables as described above for forests. Most of the SEMs showed an adequate fit between data and model (Table S2) with a mean explained variance of $P_{\text{mic}}$ of 34 % (median = 26 %) for Model 1 (Figure 2) and 25 % (median = 20 %) for Model 2 (Figure 3). For the models across all regions (ALL: Figs 2, 3), we found a significant influence of plant diversity on $P_{\text{mic}}$ concentrations in soils. If the regions were analysed individually, however, this direct effect of plant diversity was no longer significant (ALB, HAI, SCH: Figs. 2, 3). For Model 1 in which plant diversity was considered as dependent on $C_{\text{org}}$ concentrations and in case of ALL, ALB, and HAI (Figure 2), $C_{\text{org}}$ concentrations in soil were related to both, plant diversity and $P_{\text{mic}}$ concentrations in soil. However, plant diversity was related indirectly to $P_{\text{mic}}$ 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 $C_{\text{org}}$ concentrations which itself was an important explanatory variable of $P_{\text{mic}}$ concentrations (Figures 2, 3).

4 Discussion

4.1 Variables controlling $P_{\text{mic}}$ concentrations in soil

Variables controlling $P_{\text{mic}}$ concentrations in soil were suspected to be partly identical to those driving $C_{\text{mic}}$ concentrations in soil. Indeed, for both forests and grasslands, soil moisture, $C_{\text{org}}$ concentrations, ...
pH, and total P controlled $P_{\text{mic}}$ concentrations partly corroborating findings on factors driving $C_{\text{mic}}$ 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). Our results highlight the importance of soil moisture for $P_{\text{mic}}$ concentrations. Increasing soil moisture results in an increasing diffusivity of $P$ enhancing the uptake by microbes and plants (Lambers et al., 2006). In our study, soil moisture was related inversely to soil texture (Table S1). 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, we found a relationship between soil texture and $P_{\text{mic}}$ concentrations as well (Table 2). Because of the experimental design (highest moisture linked to finest texture and lowest soil moisture linked to coarsest texture), we were not able to tease apart the effects of the two variables. In line with the effect of $C_{\text{org}}$, phospholipid fatty acids (forest site: Herold et al., 2014b) and enzyme activities (grassland site: Boeddinghaus et al., 2015) were mainly related to $C_{\text{org}}$ concentrations. Soil pH effects were as strong as those of $C_{\text{org}}$ concentrations (Table 2). 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 $P_{\text{mic}}$ concentrations in soil (Table 2). This indicates $P$ immobilization in microbial biomass, suggesting $P_{\text{mic}}$ to act as a sink rather than a source of plant-available $P$ in our study. Interestingly, relationships between $P$ fractions and $P_{\text{mic}}$ concentrations in soil were closer in forests as compared to grasslands (Table 2). In conjunction with higher $P_{\text{mic}}$ concentrations (Figure 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.
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 $P_{mic}$ concentrations, respectively (Table 2). Though one must keep the collinearity of $C_{org}$ and total N concentrations in soil in mind, the greater relevance of N for microbial biomass ($C_{mic}$) in forests was already suggested by Wardle (1992, 1998). Based on our results the prominent role of N in forest soils is transferable to $P_{mic}$ concentrations. Soil C : N ratios correlated significantly with $P_{mic}$ concentrations in forest soils which was not the case for grasslands (Table 2) 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, we could accept our hypothesis namely that $C_{org}$ concentrations and soil moisture influence $P_{mic}$ concentrations in forest and grassland soils. In addition, soil pH was a strong control of $P_{mic}$ concentrations in both systems. Finally, total N concentrations, soil C : N ratios, and P fractions (except for NaOH-P) were particularly decisive for $P_{mic}$ concentrations in forest soils.

In forest soils, $P_{mic}$ concentrations increased with an increasing plant diversity (Table 2) 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 $P_{mic}$ resulted from simultaneous and positive relationships of $C_{org}$ concentrations on both plant diversity and $P_{mic}$ in grassland soils. However, this applied for mineral soil types in ALB and HAI only as high $C_{org}$ concentrations in soil associated with organic soil types such as fens in SCH (Herold et al., 2014c) revealed no link between plant diversity and $C_{org}$ or $P_{mic}$ concentrations in grassland soil. For the mineral soil types in the ALB and HAI, we suggest that the effect of plant species diversity on $P_{mic}$ 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 $P_{mic}$. 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 $C_{org}$ 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 our study. Our results suggest that the positive relationship between OM accumulation and plant diversity might be transferable from artificial grassland mixtures to established grasslands thus, confirming our hypothesis that plant diversity has an effect on Pmic concentrations in addition to soil properties.

4.2 Regional differences and management effects

In general, Pmic concentrations were higher in grassland than in forest soils (Figure 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 our 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 we did not measure Pmic 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 2). This trend corresponds to the results of total phospholipid fatty acids in grassland soils by Herold et al. (2014b). Higher P$_{\text{mic}}$ 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 Chapter 4.1, 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 P$_{\text{o}}$ and total P concentrations, and ii) higher soil pH in grassland and forest soils as compared to SCH (Alt et al., 2011). Therefore, we could confirm our hypothesis on region-specific P$_{\text{mic}}$ concentrations in soil which are caused by differences in environmental conditions among regions (C$_{\text{org}}$ as substrate availability for microorganism metabolism, pH, soil moisture).

Analysing the three regions separately, neither tree species nor management (managed versus near-natural, Table 3) had significant effects on P$_{\text{mic}}$ concentrations in forest soils. This might be caused by the fact that variables identified as controlling P$_{\text{mic}}$ concentrations (see Chapter 4.1) did not show an effect either. For our study sites, no differences in C$_{\text{org}}$ 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 P$_{\text{mic}}$ concentrations in soil (see Chapter 4.1) 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); $P < 0.05$). We infer that the important role of pH for P$_{\text{mic}}$ 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* versus *Quercus suber*) on \( P_{\text{mic}} \) concentrations in soil went along with differences in soil pH. Furthermore, we analysed mineral soil, but not the organic layer. Particularly in the organic layer, microbial properties (\( C_{\text{mic}}, N_{\text{mic}}, C_{\text{mic}}:C_{\text{org}} \) ratio, \( N_{\text{mic}}:\text{total } N \) 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, \( C_{\text{mic}} \)) 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 \( P_{\text{mic}} \) concentrations in our samples which – identical with the study of *Zhong* and *Makeschin* (2006) – were collected at a depth of 0 – 10 cm. In summary, we cannot unambiguously reject our hypotheses on tree-species/land-use intensity effects on \( P_{\text{mic}} \) concentrations in forest soils.

In accordance with results from forest soils, we found no effect of land-use types (pasture, mown pasture, meadow) or management measures (fertilization, cutting) on \( P_{\text{mic}} \) 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, \( P_{\text{mic}} \), by loss of soil due to erosion is minimized. *Bristow* and *Jarvis* (1991) found no difference between \( C_{\text{mic}} \) 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 \( C_{\text{org}} \) availability in soil – an explanation that we consider reasonable.
given the generally high soil OM concentrations in grassland ecosystems. Similarly, we found no effect of fertilization on $P_{\text{mic}}$ concentrations which contrasts with the study by Liebisch et al. (2014).

In established ecosystems such as those we studied 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 $P_{\text{mic}}$ 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 $P_{\text{mic}}$ concentrations in grassland soils. Regrettably, such information is hardly available at all or if so associated with high uncertainties. Concluding, we have to reject our hypothesis of a positive effect of land-use intensity and fertilization on $P_{\text{mic}}$ concentrations in soil.

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, $P_{\text{mic}}$ concentrations were closely related to substrate availability (especially $C_{\text{org}}$) in both, forest and grassland soils. Despite management effects on P fractions in soil and a relationship between selected P fractions and $P_{\text{mic}}$ concentrations in soil, $P_{\text{mic}}$ was found to be insensitive to forest and grassland management measures.

Acknowledgements

We thank the managers of the three Exploratories, Kirsten Reichel-Jung, Swen Renner, Katrin Hartwich, Sonja Gockel, Kerstin Wiesner, and Martin Gorke for their work in maintaining the plot and project infrastructure; Christiane Fischer and Simone Pfeiffer for giving support through the central office, Michael Owonibi for managing the central database, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser, and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We
thank Ulli Bange, Theresa Klötzung, and Doreen Berner for laboratory work and Fabian Alt and all
other members of the joint sampling campaign in May 2011 for their help.

The work has been (partly) funded by the DFG Priority Program 1374 “Infrastructure-Biodiversity-
Exploratories” (DFG-Oe516/1-2). Field work permits were issued by the responsible state
environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to
§ 72 BbgNatSchG).

References


and forest soils of Germany as related to land-use type, management intensity, and land use-

Aponte, C., Garcia, L. V., Maranon, T. (2013): Tree species effects on nutrient cycling and soil biota: A


Baumann, K., Schöning, I., Schrumpf, M., Ellerbrock, R. H., Leinweber, P. (2016): Rapid assessment of
soil organic matter: Soil color analysis and Fourier transform infrared spectroscopy.

Geoderma 278, 49.

use intensity modifies spatial distribution and function of soil microorganisms in grasslands.

Pedobiologia 54, 341-351.

Bergkemper, F., Bünemann, E. K., Hauenstein, S., Heuck, C., Kandeler, E., Krüger, J., Marhan, S.,
Mészáros, É., Nassal, D., Nassal, P., Oelmann, Y., Pistocchi, C., Schloter, M., Spohn, M.


Table 1: Main geographical and environmental characteristics of the study regions (*Fischer* et al., 2010).

<table>
<thead>
<tr>
<th>region</th>
<th>Schwäbische Alb</th>
<th>Hainich-Dün</th>
<th>Schorfheide-Chorin</th>
</tr>
</thead>
<tbody>
<tr>
<td>abbreviation</td>
<td>ALB</td>
<td>HAI</td>
<td>SCH</td>
</tr>
<tr>
<td>located in Germany</td>
<td>south-west</td>
<td>central</td>
<td>north-east</td>
</tr>
<tr>
<td>altitude</td>
<td>460 – 860 m a.s.l.</td>
<td>258 – 550 m a.s.l.</td>
<td>3 – 140 m a.s.l.</td>
</tr>
<tr>
<td>mean temperature</td>
<td>6 – 7 °C</td>
<td>6.5 – 8 °C</td>
<td>8 – 8.5 °C</td>
</tr>
<tr>
<td>mean precipitation</td>
<td>700 – 1,000 mm</td>
<td>500 – 800 mm</td>
<td>500 – 600 mm</td>
</tr>
<tr>
<td>parent material</td>
<td>calcareous bedrocks with karst phenomena</td>
<td>calcareous bedrocks of Triassic limestone covered by loess</td>
<td>glacial till covered with glacio-fluvial or Aeolian sand</td>
</tr>
<tr>
<td>soil texture</td>
<td>rich in clay</td>
<td>loamy or clayey</td>
<td>sandy loam to pure sand</td>
</tr>
<tr>
<td>soil type</td>
<td>Cambisols, Leptosols</td>
<td>Cambisols, Stagnosols, Vertisols, Luvisols</td>
<td>Histosols, Luvisols, Gleysoils, Albeluvisols, Cambisols, Regosols, Podzols</td>
</tr>
<tr>
<td>main tree species</td>
<td>beech, spruce</td>
<td>beech, spruce</td>
<td>beech, oak, pine</td>
</tr>
<tr>
<td>land-use type</td>
<td><strong>grassland:</strong> meadow, mown pasture (n = 22), pasture (n = 9)</td>
<td><strong>grassland:</strong> meadow, mown pasture (n = 7), pasture (n = 20)</td>
<td><strong>grassland:</strong> meadow, mown pasture (n = 18), pasture (n = 10), selection cutting (n = 22)</td>
</tr>
<tr>
<td></td>
<td><strong>forest:</strong> age class forest (n = 46), unmanaged forest (n = 19)</td>
<td><strong>forest:</strong> age class forest (n = 28), unmanaged forest (n = 13)</td>
<td><strong>forest:</strong> age class forest (n = 45), unmanaged forest (n = 5)</td>
</tr>
</tbody>
</table>
Table 2: Relationship of different biotic and abiotic variables to $P_{nic}$ in forest and grassland soils

$r = Pearson's$ rank correlation, $n = sample$ size; ns = not significant, $* P < 0.05$ ** $P < 0.01$, *** $P < 0.001$).

<table>
<thead>
<tr>
<th>land-use type</th>
<th>forest soils</th>
<th>grassland soils</th>
</tr>
</thead>
<tbody>
<tr>
<td>variable</td>
<td>$r$</td>
<td>$n$</td>
</tr>
<tr>
<td>clay</td>
<td>0.83***</td>
<td>133</td>
</tr>
<tr>
<td>medium silt</td>
<td>0.33***</td>
<td>133</td>
</tr>
<tr>
<td>medium sand</td>
<td>-0.58***</td>
<td>133</td>
</tr>
<tr>
<td>soil moisture</td>
<td>0.80***</td>
<td>133</td>
</tr>
<tr>
<td>(10 cm below surface)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaHCO$_3$-$P_i$</td>
<td>-0.37***</td>
<td>117</td>
</tr>
<tr>
<td>NaHCO$_3$-$P_o$</td>
<td>-0.51***</td>
<td>117</td>
</tr>
<tr>
<td>NaOH-$P_i$</td>
<td>ns</td>
<td>117</td>
</tr>
<tr>
<td>NaOH-$P_o$</td>
<td>0.57***</td>
<td>117</td>
</tr>
<tr>
<td>HCl-$P$</td>
<td>0.71***</td>
<td>117</td>
</tr>
<tr>
<td>$H_2$SO$_4$-$P$</td>
<td>0.71***</td>
<td>117</td>
</tr>
<tr>
<td>$\Sigma$ NaHCO$_3$-$P_i$ + NaOH-$P_i$</td>
<td>ns</td>
<td>117</td>
</tr>
<tr>
<td>$\Sigma$ NaHCO$_3$-$P_o$ + NaOH-$P_o$</td>
<td>0.48***</td>
<td>117</td>
</tr>
<tr>
<td>total $P$</td>
<td>0.72***</td>
<td>117</td>
</tr>
<tr>
<td>pH</td>
<td>0.72***</td>
<td>118</td>
</tr>
<tr>
<td>$C_{org}$</td>
<td>0.65***</td>
<td>133</td>
</tr>
<tr>
<td>total $N$</td>
<td>0.86***</td>
<td>133</td>
</tr>
<tr>
<td>$C : N$</td>
<td>-0.55***</td>
<td>133</td>
</tr>
<tr>
<td>$N_{nic}$</td>
<td>0.71***</td>
<td>132</td>
</tr>
<tr>
<td>$C_{mic}$</td>
<td>0.86***</td>
<td>132</td>
</tr>
<tr>
<td>$C_{mic} : N_{mic}$</td>
<td>-0.26**</td>
<td>132</td>
</tr>
<tr>
<td>plant diversity</td>
<td>0.45***</td>
<td>132</td>
</tr>
</tbody>
</table>
Table 3: 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]).

<table>
<thead>
<tr>
<th>Land-use type</th>
<th>Region</th>
<th>Schwäbische Alb</th>
<th>Hainich-Dün</th>
<th>Schorfheide-Chorin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spruce</td>
<td>48.7[±19.7]</td>
<td>29.9[±8.6] a</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>oak</td>
<td>na</td>
<td>na</td>
<td>4.8[±1.4] ab</td>
<td></td>
</tr>
<tr>
<td>pine</td>
<td>na</td>
<td>7.2[±2.9] a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age class forest</td>
<td>51.2[±18.8]</td>
<td>24.6[±10.7] a</td>
<td>5.8[±2.9] a</td>
<td></td>
</tr>
<tr>
<td>unmanaged forest</td>
<td>66.0[±13.3]</td>
<td>24.5[±8.9] ab</td>
<td>6.7[±3.7] a</td>
<td></td>
</tr>
<tr>
<td>selection cutting</td>
<td>na</td>
<td>15.6[±2.7] b</td>
<td>na</td>
<td></td>
</tr>
<tr>
<td>Fertilization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>63.4[±24.1]</td>
<td>49.7[±24.2] a</td>
<td>46.0[±26.5] a</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>66.3[±20.7]</td>
<td>52.7[±14.7] a</td>
<td>42.7[±32.0] a</td>
<td></td>
</tr>
<tr>
<td>Grassland soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>meadow</td>
<td>64.2[±23.7]</td>
<td>44.2[±25.1] a</td>
<td>53.1[±29.3] a</td>
<td></td>
</tr>
<tr>
<td>mown pasture</td>
<td>64.6[±25.7]</td>
<td>52.9[±22.0] a</td>
<td>52.2[±35.5] a</td>
<td></td>
</tr>
<tr>
<td>pasture</td>
<td>65.1[±21.0]</td>
<td>52.0[±15.6] a</td>
<td>31.3[±26.3] a</td>
<td></td>
</tr>
<tr>
<td>Number of cuts per year</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>66.1[±21.0]</td>
<td>52.6[±14.8] a</td>
<td>31.3[±26.3] a</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70.5[±22.5]</td>
<td>54.1[±25.4] a</td>
<td>46.7[±35.2] a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>64.8[±25.6]</td>
<td>39.6[±8.5] a</td>
<td>60.0[±25.1] a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>51.7[±18.9]</td>
<td>26.3[±5.1] a</td>
<td>58.7[±30.2] a</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Mean concentrations of P$_{\text{mic}}$ 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 [$P < 0.05$]).

Figure 2: Structural equation model 1 of the relationships between different abiotic controlling factors, plant diversity (diversity) and P$_{\text{mic}}$ in grassland sites for ALL (all three regions together, RMSEA = 0.081), ALB (Schwäbische Alb, RMSEA < 0.001), HAI (Hainich-Dün, RMSEA < 0.001) and SCH (Schorfheide-Chorin, RMSEA < 0.001). In this model, plant diversity is considered as response variable i.e., depending on organic C (C$_{\text{org}}$) 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: Structural equation model 2 of the relationships between different abiotic controlling factors, plant diversity (diversity) and P$_{\text{mic}}$ in grassland sites with plant diversity as driving factor for ALL (all three regions together, RMSEA = 0.271), ALB (Schwäbische Alb, RMSEA < 0.001), HAI (Hainich-Dün, RMSEA = 0.180), and SCH (Schorfheide-Chorin, RMSEA < 0.001). In this model, plant diversity is considered as independent variable i.e., controlling both organic C (C$_{\text{org}}$) and P$_{\text{mic}}$ 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 1:

![Bar chart showing microbial P (mg kg⁻¹) at forest site and grassland site](image-url)
Figure 2: 

[Diagram showing the relationships between total P, C$_{org}$, diversity, and P$_{mic}$ in different soil types (ALL, ALB, HAI, SCH).]
Figure 3: