Doctoral Thesis

Phosphorus dynamics in a ferralsol under maize-fallow rotations: the role of the soil microbial biomass

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PHOSPHORUS DYNAMICS IN A FERRALSOL UNDER MAIZE-FALLOW ROTATIONS: THE ROLE OF THE SOIL MICROBIAL BIOMASS

A dissertation submitted to the

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presented by

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2003
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BNF</td>
<td>biological nitrogen fixation</td>
</tr>
<tr>
<td>Ca&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>exchangeable calcium (extractable with 1 M KCl)</td>
</tr>
<tr>
<td>C&lt;sub&gt;chl&lt;/sub&gt;</td>
<td>chloroform-labile (microbial) C</td>
</tr>
<tr>
<td>CEC</td>
<td>cation exchange capacity</td>
</tr>
<tr>
<td>C&lt;sub&gt;H2O&lt;/sub&gt;</td>
<td>water-soluble C</td>
</tr>
<tr>
<td>C&lt;sub&gt;l&lt;/sub&gt;</td>
<td>labile C (oxidized by 0.333 M KMnO&lt;sub&gt;4&lt;/sub&gt;)</td>
</tr>
<tr>
<td>COM</td>
<td>continuous maize</td>
</tr>
<tr>
<td>cp</td>
<td>concentration of inorganic P in the soil solution</td>
</tr>
<tr>
<td>C&lt;sub&gt;tot&lt;/sub&gt;</td>
<td>total C</td>
</tr>
<tr>
<td>15N</td>
<td>isotopic composition of N in relation to atmospheric N&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>E&lt;sub&gt;1min&lt;/sub&gt;</td>
<td>amount of P isotopically exchangeable within 1 min</td>
</tr>
<tr>
<td>EA</td>
<td>exchangeable acidity (extractable with 1 M KCl)</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;dest&lt;/sub&gt;</td>
<td>distilled water</td>
</tr>
<tr>
<td>HSD&lt;sub&gt;0.05&lt;/sub&gt;</td>
<td>honestly significant difference after Tukey’s multiple range test (&lt;i&gt;P&lt;/i&gt; = 0.05)</td>
</tr>
<tr>
<td>ICRAF</td>
<td>International Centre for Research in Agroforestry</td>
</tr>
<tr>
<td>K&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>exchangeable K (extractable with 0.5 M NaHCO&lt;sub&gt;3&lt;/sub&gt; + 0.01 M EDTA)</td>
</tr>
<tr>
<td>LR</td>
<td>long rainy season</td>
</tr>
<tr>
<td>MCF</td>
<td>maize-crotalaria fallow rotation</td>
</tr>
<tr>
<td>Mg&lt;sub&gt;ex&lt;/sub&gt;</td>
<td>exchangeable Mg (extractable with 1 M KCl)</td>
</tr>
<tr>
<td>MNF</td>
<td>maize-natural fallow rotation</td>
</tr>
<tr>
<td>n</td>
<td>rate of disappearance of radioactivity from the solution for &gt; 1 min</td>
</tr>
<tr>
<td>NaOH-P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>inorganic P extractable with 0.1 M NaOH</td>
</tr>
<tr>
<td>NaOH-P&lt;sub&gt;o&lt;/sub&gt;</td>
<td>organic P extractable with 0.1 M NaOH</td>
</tr>
<tr>
<td>NaOH-P&lt;sub&gt;resin&lt;/sub&gt;</td>
<td>P extractable with 0.1 M NaOH that is recovered on resin-membranes</td>
</tr>
<tr>
<td>NaOH-P&lt;sub&gt;1&lt;/sub&gt;</td>
<td>total P extractable with 0.1 M NaOH</td>
</tr>
<tr>
<td>N&lt;sub&gt;chl&lt;/sub&gt;</td>
<td>chloroform-labile (microbial) N</td>
</tr>
<tr>
<td>N&lt;sub&gt;K2SO4&lt;/sub&gt;</td>
<td>total N extractable with 0.5 M K&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>N&lt;sub&gt;min&lt;/sub&gt;</td>
<td>mineral N (sum of ammonium and nitrate) extractable with 2 M KCl</td>
</tr>
<tr>
<td>ns</td>
<td>not significant</td>
</tr>
<tr>
<td>N&lt;sub&gt;tot&lt;/sub&gt;</td>
<td>total N</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>$P$</td>
<td>probability level</td>
</tr>
<tr>
<td>$P_{\text{Bray}}$</td>
<td>$P$ extractable by Bray-1 method ($0.03, M, \text{NH}_4\text{F} + 0.025, M, \text{HCl}$)</td>
</tr>
<tr>
<td>$P_{\text{fum}}$</td>
<td>$P$ extracted from fumigated samples</td>
</tr>
<tr>
<td>$P_{\text{hex}}$</td>
<td>hexanol-labile (microbial) $P$</td>
</tr>
<tr>
<td>$P_{\text{H}_2\text{SO}_4}$</td>
<td>$P$ extracted with $0.5, M, \text{H}_2\text{SO}_4$ from non-ignited soils</td>
</tr>
<tr>
<td>$p\text{H}_{\text{H}_2\text{O}}$</td>
<td>$pH$ measured in water</td>
</tr>
<tr>
<td>$P_{\text{i}}$</td>
<td>inorganic $P$</td>
</tr>
<tr>
<td>PLFA</td>
<td>phospholipid fatty acid</td>
</tr>
<tr>
<td>$P_{\text{o}}$</td>
<td>organic $P$</td>
</tr>
<tr>
<td>$P_{\text{Olsen}}$</td>
<td>$P$ extractable by modified Olsen method ($0.5, M, \text{NaHCO}_3 + 0.01, M, \text{EDTA}$)</td>
</tr>
<tr>
<td>$P_{\text{resin}}$</td>
<td>resin-extractable $P$</td>
</tr>
<tr>
<td>$P_{\text{tot}}$</td>
<td>total $P$</td>
</tr>
<tr>
<td>$q\text{CO}_2$</td>
<td>metabolic quotient (ratio of respiration and microbial $C$)</td>
</tr>
<tr>
<td>$R$</td>
<td>total introduced radioactivity</td>
</tr>
<tr>
<td>$r_t$</td>
<td>radioactivity remaining in the solution after $t$ minutes of isotopic exchange</td>
</tr>
<tr>
<td>SA</td>
<td>specific activity ($^{33}\text{P}/^{31}\text{P}$)</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SED</td>
<td>standard error of the difference in means</td>
</tr>
<tr>
<td>soil IEP</td>
<td>isotopically exchangeable soil $P$</td>
</tr>
<tr>
<td>SR</td>
<td>short rainy season</td>
</tr>
<tr>
<td>TSP</td>
<td>triple superphosphate</td>
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</table>
Abstract

Microbial processes in the dynamics of phosphorus (P) in highly weathered soils of the tropics are not well understood. In this thesis, the introduction of a one-season fallow with the legume *Crotalaria grahamiana* into continuous maize cultivation (with two harvests per year) on a Ferralsol in western Kenya was chosen as a model case to investigate how the soil microbial biomass affects plant available P. Maize and fallow productivity and changes in soil properties during 5.5 years were studied in a field experiment with three crop rotations (continuous maize, maize-crotalaria fallow and maize-natural fallow rotation) at two levels of P fertilization (0 and 50 kg P ha\(^{-1}\), applied as triple superphosphate). Soil samples from the field were also used in incubation and pot experiments.

Total maize production did not differ significantly among crop rotations, indicating that the maize yield forgone during the fallow season was compensated by higher post-fallow yields. During the first fallow season, the crotalaria fallow was more productive than the natural fallow, but due to pest problems, crotalaria growth decreased in the course of the experiment. In all crop rotations, P fertilization doubled total maize yields and resulted in positive P balances, but did not affect amounts of recycled biomass. N balances were negative in all cases. Both fallow types reversed the trend for soil organic matter losses observed under continuous maize. Highest levels of soil organic and microbial C, N and P were found in the maize-crotalaria rotation, while available inorganic P was similar in all crop rotations. Maize-legume fallow rotations complemented by balanced additions of inorganic fertilizers can improve yields and prevent soil degradation.

The composition of the microbial community as indicated by phospholipid fatty acid analysis differed between soils from continuous maize and maize-crotalaria rotation. Higher amounts of microbial biomass were connected with an increase in the relative abundances of indicators for fungi and gram-negative bacteria. P fertilization affected the community profile only in soils under continuous maize. In incubation experiments, the decomposition of glucose, cellulose and three plant residues proceeded faster in soil from the maize-crotalaria rotation, but differences were mostly transient. Microbial P and N uptake within one week increased together with the proportion of water-soluble C
in added plant residues and with the initial size of the microbial biomass. P fertilization had a limited effect on decomposition rates and microbial P uptake, confirming field observations that the microorganisms are limited by C and N rather than P availability.

The cycling of P from different sources (added inorganic P, plant residue P, and soil P) labeled with $^{33}\text{P}$ was investigated during nine weeks of incubation. On average, 15% of $^{33}\text{P}$ from the plant residue was recovered in the microbial biomass, compared to 7 and 4% after carrier-free labeling and addition of inorganic P, respectively. More than half of the increase in microbial P after residue addition was derived from the soil. Microbial uptake of $^{33}\text{P}$ from all P sources appeared to occur mainly within the first day after soil amendment and remained almost unchanged thereafter, whereas the recovery of $^{33}\text{P}$ in resin-extractable P declined steadily from 7-22% after one day, depending on the P source, to 3-5% after nine weeks. Maize growing on the same soils took up a maximum of 2% of $^{33}\text{P}$ during growth periods of three weeks, with a higher recovery from added inorganic P than from the other two sources.

The microbial biomass is suggested to represent a very dynamic P compartment in a highly weathered soil, as the presence of labile C induces pronounced patterns of P immobilization and re-mineralization. To manage resulting adverse or beneficial effects on P availability to plants, a better understanding of microbial dynamics is still required.

Zusammenfassung

nur in Böden unter Maisdaueranbau. In Inkubationsexperimenten verlief der Abbau von Glucose, Cellulose und drei Pflanzenrückständen schneller im Boden aus der Mais-Crotalariabrache, aber Unterschiede bestanden meistens nur vorübergehend. Die mikrobielle Aufnahme von P und N innerhalb einer Woche nahm zu mit dem Anteil an wasserlöslichem C in den zugegebenen Pflanzenrückständen sowie mit der Anfangsgröße der mikrobiellen Biomasse. P-Düngung hatte einen begrenzten Einfluss auf die Abbauraten und die mikrobielle P-Aufnahme, wodurch Beobachtungen aus dem Feld bestätigt wurden, dass die Mikroorganismen stärker durch C und N limitiert sind als durch P.

Der P-Fluss aus verschiedenen, mit \(^{33}\text{P}\) markierten Quellen (zugegebenes anorganisches P, P aus einem Pflanzenrückstand, und Boden-P) wurde während einer neunwöchigen Inkubation untersucht. Durchschnittlich 15% des \(^{33}\text{P}\) aus dem Pflanzenrückstand und 4% aus zugegebenem anorganischem P wurde in der mikrobiellen Biomasse wiedergefunden, verglichen mit 7% nach Markierung des Bodens mit \(^{33}\text{P}\). Mehr als die Hälfte des Anstiegs in mikrobiellem P nach Zugabe des Pflanzenrückstands stammte aus dem Boden. Die mikrobielle Aufnahme von \(^{33}\text{P}\) aus allen Quellen lief offenbar hauptsächlich innerhalb des ersten Tages nach Zugabe zum Boden ab und blieb danach fast unverändert, während die Wiederfindung von \(^{33}\text{P}\) in harzextrahierbarem P fortlaufend von – je nach P-Quelle – 7-22% nach einem Tag auf 3-5% nach neun Wochen abnahm. In dreiwöchigen Wachstumsperioden nahm Mais von denselben Böden maximal 2% des \(^{33}\text{P}\) auf, wobei die Wiederfindung aus zugegebenem anorganischem P grösser war als aus den beiden anderen P-Quellen.

Es wird vorgeschlagen, dass die mikrobielle Biomasse ein sehr dynamisches P-Kompartiment in einem stark verwitterten Boden darstellt, da die Anwesenheit von labilem C ausgeprägte Phasen von Immobilisierung und Remineralisierung auslöste. Um daraus resultierende negative oder positive Auswirkungen auf die P-Verfügbarkeit für Pflanzen steuern zu können, muss das Verständnis mikrobieller Dynamik noch verbessert werden.
General introduction
**Biological importance of phosphorus**

In nature, phosphorus (P) occurs mainly as orthophosphate (PO$_4^{3-}$) bound to different compounds. P is an essential element for all living cells. As a component of nucleic acids (DNA and RNA), phospholipids, sugar phosphates (e.g. glucose-6-phosphate) and molecules with an energy-rich pyrophosphate bond (e.g. ATP), it is involved in carrying genetic information and translating it for protein synthesis, in creating biological compartments separated by semi-permeable biomembranes, and in providing a form of energy transfer for physiological metabolism. In addition, inorganic phosphate (P$_i$) controls several enzyme reactions. In the vegetative tissue of higher plants, most P$_i$ is therefore located in the vacuole. In pollen, seeds and tubers, phytates (salts of phytic acid or myoinositol) are the major storage form of P. Storage of P in inorganic polyphosphates has also been reported for plants, but is more widespread among microorganisms (Marschner, 1995).

P deficiency depresses plant growth. The leaf area of the plant is significantly reduced, while the chlorophyll content is usually not affected or even increased, making the darker green color of leaves a typical symptom for P deficiency (Marschner, 1995). From an agronomic perspective, the strong reduction in the formation of reproductive organs under P deficiency makes adequate P supply to plants an important condition to achieve sufficient crop yields.

**Forms of P in soil**

In soils, P occurs in inorganic and organic forms. Apatite, the most common primary P mineral, is dissolved in the presence of H$^+$. Orthophosphate in solution can then precipitate with Ca, Al or Fe to subsequently form various secondary P minerals, be sorbed on surfaces in a rapid exothermic followed by a slow endothermic reaction, or taken up by soil organisms and plants (Frossard et al., 1995). All soil organic P (P$_o$) is derived from organisms, but due to selective stabilization of some forms of P$_o$, the composition of P$_o$ differs between living cells and soils (Magid et al., 1996). While the majority of P$_o$ in growing organisms is in nucleic acids, monoesters such as inositol hexaphosphate often represent more than 50% of P$_o$ extractable from soils. Other identified forms of P$_o$ in soils include diesters such as phospholipids and nucleic acids, and phosphonates (C–P bond).
Duration and intensity of weathering affect forms of soil P, with P in primary minerals and Ca phosphates decreasing during pedogenesis. At the same time, the amounts of P in secondary minerals and in \( P_0 \) increase. In highly weathered soils, the proportion of \( P_0 \) is usually greater than in young soils, especially in relation to labile \( P_i \). This conceptual model developed by Walker and Syers (1976) from soil sequences in New Zealand has been confirmed for a chronosequence in Hawaii (Crews et al., 1995) and by a literature review of soil P fractions in natural ecosystems (Cross and Schlesinger, 1995).

**Availability of soil P to plants**

Plants take up P as hydrogenated orthophosphate ions (\( H_2PO_4^- \) or \( HPO_4^{2-} \)) from the soil solution in an active, energy dependent process. A water film around soil particles is thus required for roots to take up P. Due to the high reactivity of P with the solid phase, less than 0.1% of total soil P is usually found in the soil solution (Fardeau, 1996). Phosphate ions released from the solid phase by desorption or dissolution and \( P_i \) released from \( P_0 \) by mineralization also contribute to P that can be taken up by plants. Thus, both physicochemical and biological processes are involved in bringing \( P_i \) to the soil solution. In addition, plants have developed different strategies to acquire P, including greater root (hair) length and density, symbiosis with mycorrhizal fungi, and root exudation of phosphatase enzymes, organic acids, and protons (Rao et al., 1999). Root branching and root hair development can also be affected by phytohormones produced by microorganisms (Holguin et al., 1999). In the following sections, only the soil-dependent processes and their measurement will be described.

**Physicochemical processes determining soil P availability**

The physicochemical processes determining P availability are described by three factors (Beckett and White, 1964): the intensity factor (the concentration of \( P_i \) in the soil solution), the quantity factor (the amount of P that can be released from the solid phase to the soil solution) and the buffer capacity (the variation in the intensity factor when the quantity factor varies due to plant P uptake or P inputs). With chemical extraction methods, a quantity factor is determined which usually contains a fraction of unavailable soil P while at the same time, not all available P is captured (Fardeau, 1996). The buffer capacity is usually determined with P sorption isotherms (Fox and
General introduction

Kamprath, 1970), which describe the relationship between the concentration of Pt in the soil solution and the amount of P sorbed by the soil. With isotopic exchange kinetics, all three factors are determined: the concentration of Pt in the soil solution (cp) represents the intensity factor, the quantity factor is calculated as the amount of Pt which is isotopically exchangeable within different times, and the buffering capacity is related to the fraction of the total introduced radioactivity (R) remaining in the soil solution after one minute (r1) and to the parameter n which describes the rate of disappearance of the tracer from the solution for longer times (Fardeau, 1996; Frossard and Sinaj, 1997).

Biological processes determining soil P availability

Biological processes may also have a significant influence on P availability in soils. Similar to concepts developed for N mineralization (Mary and Recous, 1994), net mineralization of P₀ is the result of several processes that can occur simultaneously: biological immobilization, re-mineralization during the decline of a microbial population, constant basal mineralization of soil P₀, and flush effects due to soil drying and rewetting or freezing and thawing events (Oehl et al., 2001b).

Free-living soil organisms belong to the bacteria, fungi, algae, and soil animals, while viruses grow only within living cells of other organisms (Paul and Clark, 1988). In addition, plant roots are sometimes included in the soil biota (Killham, 1994). Bacteria and fungi have been estimated to represent more than 99% of the amount of P contained in living cells in the soil, excluding roots (Magid et al., 1996). While protozoa, nematodes and other members of the soil fauna contribute little to the total dry weight and P content of soil organisms, they may contribute to the control of microbial P immobilization, in addition to the control by climatic factors and substrate availability. For example, amoebal grazing of bacteria caused a release of Pt in soil microcosms (Cole et al., 1978).

The amount of P in the microbial biomass is determined with modifications of the fumigation-extraction method (Brookes et al., 1982; Hedley and Stewart, 1982), depending on the soil, and is usually at least similar to the amount of P in above-ground biomass of annual crops and pastures (Richardson, 1994). Changes in the composition of the microbial community can be revealed by phospholipid fatty acid analysis
(PLFA), comparing the community profiles of these membrane-bound fatty acids (Zelles, 1999).

Besides constituting a labile reservoir of P, soil microorganisms mediate P transformations. Many soil bacteria and fungi have been shown to solubilize Ca, Fe and Al phosphates in laboratory assays (Kucey et al., 1989). Mineralization of P<sub>0</sub> requires the activity of phosphatase enzymes which are produced by soil microorganisms as well as plants. After secretion or cell lysis, phosphatases may be adsorbed on surfaces, and the separation of extracellular enzyme activities from those associated with living organisms is difficult (Tabatabai, 1994). Phosphatase activity is related to total microbial activity and is decreased by P fertilization (Richardson, 1994). The availability of P<sub>i</sub> and the P demand of microorganisms and plants may govern P<sub>0</sub> mineralization, but P<sub>i</sub> may also be released from soil organic matter as a by-product of C mineralization to satisfy energy requirements of the microbial biomass (Stewart and Tiessen, 1987). Therefore, both phosphatase activity and soil respiration have been used as indicators of P<sub>0</sub> mineralization (Oberson et al., 2001). The direct measurement of P<sub>0</sub> mineralization poses difficulties, as any released P<sub>i</sub> will react rapidly with the solid phase, precluding the use of extraction or leaching of P from soils after various times of incubation similar to the measurement of N mineralization. Therefore, isotopic dilution methods have been developed which are based on the assumption that P<sub>i</sub> released from P<sub>0</sub> will decrease the specific activity of isotopically exchangeable P<sub>i</sub> in a soil labeled with ³²P (Oehl et al., 2001b).

**Soil P dynamics**

Soil P dynamics are characterized by interactions between the physicochemical and the biological processes: the immobilization of P<sub>i</sub> in microorganisms constitutes a withdrawal of P<sub>i</sub> from the soil solution (Seeling and Zasoski, 1993), while at the same time, P is delivered to the soil solution through re-mineralization of microbial P and P<sub>0</sub> mineralization. In addition, organic anions released from microorganisms, plant roots or added organic materials can affect P sorption and the exchangeability of added P through competition for sorption sites (Le Mare et al., 1987; Nziguheba et al., 1998). Oberson et al. (1999) observed less P sorption and higher levels of P<sub>0</sub> as well as labile P<sub>i</sub> under an improved grass-legume pasture in Colombia compared to a grass-only pasture,
although both systems had received similar P inputs, and P outputs were even greater from the grass-legume pasture. While this provided evidence for the role of biological activity in improving P availability to plants, especially in highly weathered soils, the methods and concepts of biological processes in soil P dynamics are less advanced than the knowledge regarding physicochemical reactions.

Soil P dynamics can be studied using P isotopes as tracers. For example, the recovery of $^{32}$P and $^{33}$P from labeled inorganic fertilizers as well as plant residues in the microbial biomass and in growing plants was compared in pot and field experiments (McLaughlin and Alston, 1986; McLaughlin et al., 1988). Carrier-free labeling of isotopically exchangeable soil $P_i$ (soil IEP) was also used to determine fluxes of $P_i$ into the microbial biomass (Oehl et al., 2001a). Soil P transformations were further investigated by combining isotopic labeling of soil IEP (Bühler et al., 2002), addition of labeled $P_i$ (Friesen and Blair, 1988) or addition of labeled plant residues (Friesen and Blair, 1988; Daroub et al., 2000) with sequential extraction after various incubation periods. Due to the short half-life of P isotopes (14.3 and 25.4 days for $^{32}$P and $^{33}$P, respectively), such studies are limited to time frames of about three months. For longer time frames, soil P dynamics are usually concluded from changes in P fractions and forms over time, between different systems or soil types.

Agronomic measures to increase soil P availability

Available $P_i$ is increased by the addition of phosphate fertilizers, even if usually less than 15% of recently applied fertilizer P is utilized by the first crop (Fardeau, 1996). Global reserves of P deposits are limited and non-renewable, and eutrophication of water bodies as a result of excessive P fertilization must be avoided. The efficient use of fertilizer as well as soil P is therefore mandatory.

Any improvement in the mobilization of soil P requires a better understanding and management of the soil biological processes. While reports on positive plant responses to microbial inoculation are frequent, studies indicating the opposite are also found. Richardson (2001) cautions that lacking or negative plant responses to microbial inoculation are less likely to be reported than positive effects, and concludes that the variable performance of microbial inoculants in soils limits their application. In addition, the indigenous microorganisms in a P-deficient soil should be well adapted to
these conditions, if microbial evolution and diversification is actively ongoing and genotypes differ between geographic locations (Tiedje et al., 2001), and should thus have developed efficient strategies to mobilize P. Consequently, the challenge is to manage the indigenous population (as well as potential inoculants) in such a way that the soil P which is mobilized by microorganisms becomes available to plants.

Plant P uptake itself also represents a mobilization of soil P, with potential benefits to the next crop if the residues are returned to the soil. Positive rotational effects which were attributed to a mobilization of soil P have been reported for several tropical legumes (Kamh et al., 2002). Besides constituting a source of P as well as other nutrients, organic materials provide an energy substrate for microbial activity, influence patterns of immobilization and re-mineralization, are precursors to soil organic matter, and may reduce P sorption of the soil (Palm et al., 1997). Due to the biological nature of most of these processes, microbial dynamics must be understood even when using plants to mobilize soil P. In addition, interactions with other nutrients, especially N, must also be considered.

Background of the study: maize-fallow rotations on Ferralsols in western Kenya

For this thesis, maize-fallow rotations on Ferralsols in western Kenya were chosen as a model case to study the role of the soil microbial biomass in the P dynamics of a highly weathered soil.

In the World Reference Base for Soil Resources, highly weathered soils of the tropics include the soil units Acrisols, Alisols, Ferralsols, Lixisols, Nitisols, and Plinthosols (FAO/ISRIC/ISSS, 1998). Due to long and intensive weathering, their mineralogy is dominated by 1:1 layer silicates of the kaolin group, sesquioxides, and other highly resistant minerals (Schwertmann and Herbillon, 1992). Under these conditions, the delivery of P onto the soil solution through physicochemical processes is low, while the importance of the biological processes in soil P dynamics depends on soil management.

Upon cultivation, a large proportion of soil organic matter is often lost from soils in the tropics within a few years (Tiessen et al., 1994), with kaolinitic soils showing the least potential to stabilize soil C (Shang and Tiessen, 1997; Wattel-Koekkoek et al., 2003). During this rapid mineralization of soil C, levels of P also decrease (Adepetu and Corey, 1977; Mueller-Harvey et al., 1985; Solomon and Lehmann, 2000). Besides being
a source of plant nutrients, soil organic matter plays an important role in soil physical properties (aggregation, water infiltration, water holding capacity), ion exchange capacity, and as the primary source of energy for most soil organisms (Craswell and Lefroy, 2001).

In the traditional system of shifting cultivation in the humid tropics, cropping periods of 1-4 years duration alternated with fallow periods of up to 15 years, during which levels of soil organic matter were largely restored by above- and belowground biomass production of woody secondary vegetation (Szott et al., 1999). Due to high population density and land scarcity, however, the duration of fallow periods in western Kenya is now often not longer than one or two growing seasons (Swinkels et al., 1997). The biomass production of such weedy fallows is too low to replenish organic matter lost during the cropping period, a situation which according to a simulation model applies to 90% of farms in western Kenya (Shepherd and Soule, 1998).

Besides inherent soil properties and limited organic matter inputs, low fertilizer inputs cause deficiency of P and other nutrients in western Kenya as well as other regions in the tropics. In the year 2000, for example, the P fertilizer consumption in Africa amounted to 0.4 million t P compared to 1.6 million t in Europe (IFA, 2003). Smaling et al. (1997) calculated average N, P and K balances for sub-Saharan Africa (except Namibia and South Africa) of -22, -2.5 and -15 kg ha\(^{-1}\) yr\(^{-1}\), respectively, with Kenya being among the countries with the highest depletion rates. According to Sanchez (2002), the price of mineral fertilizers is 2-6 times higher for farmers in Africa than in Europe, North America, or Asia, increasing several times between the seaports and towns in the interior.

One important approach used by the International Centre for Research in Agroforestry (ICRAF) during the last decade to tackle nutrient deficiencies in smallholder agriculture in sub-Saharan Africa has been the development of crop rotations that include highly productive fallows, usually consisting of planted legumes, which allow the fallow period to be shortened compared to a natural weedy fallow. While ICRAF generally refers to such fallows as ‘improved’ fallows (Sanchez, 1999), the terms ‘managed’ and ‘planted’ fallows (Szott et al., 1999; Niang et al., 2002) are also common. Under the bimodal rainfall regime in western Kenya, such fallows are relay-established in the long
rainy season’s maize crop and continue to grow during the less reliable short rainy season when maize production often fails (Sanchez, 1999).

Reported benefits of legume fallows include N inputs through biological nitrogen fixation (Gathumbi et al., 2002) and the retrieval of inorganic N from subsoil layers (Hartemink et al., 1996; Mekonnen et al., 1997). Soil samples taken 2-4 months after incorporation of fallow biomass contained higher amounts of N and P in the light fraction of soil organic matter than soils under continuous maize cultivation (Maroko et al., 1998; 1999; Smestad et al., 2002). The amount of P in soil organic matter fractions was shown to decrease again during subsequent cultivation, suggesting that P0 was mineralized (Maroko et al., 1999). Unless water availability was limiting plant growth, the grain yield of three maize crops was twice as high after fallow incorporation than after continuous maize cultivation. While this suggests improvements in nutrient availability, replenishment of P stocks via addition of inorganic fertilizer is considered necessary if soils are seriously depleted after decades of cropping without P inputs (Sanchez et al., 1997).

The yield response to improved fallows grown for <1 yr usually extends to the first subsequent crop only (Sanchez, 1999), necessitating the regular inclusion of a one-season fallow into the crop rotation. Soil P dynamics in annual maize-fallow rotations have not been documented over several years. Likewise, possible interactions between inorganic P fertilization and fallow biomass incorporation have not been investigated. In addition to the general need to test a new crop rotation for several years before large-scale adoption, nutrient input-output balances should be established and changes in nutrient pools determined in order to predict how system productivity will be affected in the long term.
Objectives, hypotheses, and structure of this thesis

The main objective of this thesis was to elucidate the role of the soil microbial biomass in P dynamics of highly weathered soils, using maize-fallow rotations on Ferralsols in western Kenya as a model case. In these rotations, maize is grown during the long rainy season, followed by fallows during the short rainy season. In collaboration with ICRAF, a field experiment was selected in which various maize-fallow rotations have been compared at different levels of P fertilization since 1997. The legume species tested in this trial was *Crotalaria grahamiana* Wight & Arn. (subsequently referred to as crotalaria). The maize-crotalaria fallow rotation was compared to two traditional crop rotations (continuous maize and maize-natural fallow rotation), all studied at two levels of P fertilization (0 and 50 kg P ha\(^{-1}\) yr\(^{-1}\), applied as triple superphosphate).

The first chapter deals with the field experiment, presenting maize and fallow productivity, N and P balances, and changes in soil properties during 5.5 years. In addition, some factors limiting microbial P uptake were investigated. The main hypothesis was that the incorporation of fallow biomass affects soil P dynamics by enhancing biological processes. P fertilization was expected to increase soil P\(_i\) availability, maize productivity, and to interact positively with the legume fallow.

The second chapter shows the results from several incubation experiments with soil samples from the field experiment, under the main hypothesis that the decomposition of added substrates as well as patterns of immobilization and re-mineralization depend on the initial properties of the soil microbial biomass. In addition to the initial amount of microbial biomass as determined with fumigation-extraction methods, the initial composition of the microbial community was also examined, using phospholipid fatty acid analysis (PLFA).

The third chapter gives a short overview of methods to measure the basal mineralization of soil P\(_o\), and presents an attempt to use isotopic dilution methods with samples from the field experiment. The hypothesis that rates of basal P\(_o\) mineralization differ between soils from the three crop rotations was tested in a pot experiment.
In the fourth chapter, the extent and temporal dynamics of microbial and plant uptake from three P sources (isotopically exchangeable soil P<sub>i</sub> (soil IEP), added P, and plant residue P) labeled with ³²P were investigated in a parallel incubation and pot experiment. It was hypothesized that the recovery of ³²P from the different sources in the microbial biomass would range in the order plant residue P > soil IEP = added P, because a net increase in microbial P would occur only after addition of a plant residue. For the plant, the recovery of ³²P was expected to range in the order added P<sub>i</sub> > plant residue P > soil IEP, reflecting the higher availability of freshly added P. Because of immobilization and re-mineralization patterns, temporal dynamics were expected to be most pronounced after addition of the plant residue. Also in this experiment, soils from the field experiment with different initial properties of the microbial biomass were used.

In the general discussion and conclusions, the results from the four chapters are synthesized. The role of the soil microbial biomass in P dynamics is discussed on the process level and for the system of maize-fallow rotations in western Kenya.
Maize productivity and nutrient dynamics in maize-fallow rotations in western Kenya

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submitted to Plant and Soil
“haste makes waste”

*English proverb*
Abstract

One-season fallows with legumes such as *Crotalaria grahamiana* Wight & Arn. and phosphorus (P) fertilization have been suggested to improve crop yields in sub-Saharan Africa. Assessing the sustainability of these measures requires a sound understanding of soil processes, especially transformations of P which is often the main limiting nutrient. We compared plant production, nitrogen (N) and P balances and selected soil properties during 5.5 years in a field experiment with three crop rotations (continuous maize, maize-crotalaria and maize-natural fallow rotation) at two levels of P fertilization (0 and 50 kg P ha\(^{-1}\) yr\(^{-1}\), applied as triple superphosphate) on a Ferralsol in western Kenya. The maize yield forgone during growth of the crotalaria fallow was compensated by higher post-fallow yields, but the cumulative total maize yield was not significantly different from continuous maize. In all crop rotations, P fertilization doubled total maize yields, increased N removal by maize and remained without effect on amounts of recycled biomass. Crotalaria growth decreased in the course of the experiment due to pest problems. Both fallow types reversed the trend for soil organic matter losses observed under continuous maize. Highest levels of soil organic and microbial C, N and P were found in the maize-crotalaria fallow rotation. The increase in organic P was not accompanied by a change in resin-extractable P, while H\(_2\)SO\(_4\)-extractable inorganic P was depleted by up to 38 kg P ha\(^{-1}\) (1% of total P) in the 0-50 cm layer. Microbial P increased substantially when soil was supplied with C and N in a laboratory experiment, confirming field observations that it is limited by C and N rather than P availability. Maize-legume fallow rotations result in a shift towards organic and microbial nutrients and have to be complemented by balanced additions of inorganic fertilizers.
Introduction

Vegetated fallows are an integral part of many tropical agroecosystems. In the traditional shifting cultivation cycle, 1-4 years of cropping alternated with up to 15 years of naturally occurring woody secondary vegetation in order to restore soil fertility and reduce weed and pest pressure (Szott et al., 1999). Even in densely populated areas such as western Kenya with 500-1200 people km\(^2\) and farm sizes of 0.5-2 ha, 52\% of farmers were found to use natural fallows, the majority thereof (59\%) for only one or two growing seasons (Swinkels et al., 1997). Due to the short duration as well as the absence of seed sources for bushes and trees, however, these fallows consist mainly of grasses and weeds, resulting in slow rates of biomass accumulation. In this situation, the deliberate planting of rapidly growing fallow species can help to shorten the required fallow period, with the additional benefit of N inputs from biological nitrogen fixation in the case of legumes (van Noordwijk, 1999). Such fallows are referred to as ‘managed’ (Szott et al., 1999), ‘planted’ (Niang et al., 2002) or ‘improved’ fallows (Sanchez, 1999).

In western Kenya, fallows with legumes such as *Sesbania sesban*, *Crotalaria grahamiana* and *Tephrosia vogelii* were able to fix up to 142 kg N ha\(^{-1}\) within 9 months under non-PK limiting conditions (Gathumbi et al., 2002). In addition, deep-rooting *Sesbania sesban* was shown to recover nitrate from the subsoil (Hartemink et al., 1996). On 80% of soils in western Kenya, however, P is the main limiting nutrient, and N fertilization without the simultaneous addition of P frequently failed to increase maize yields (Jama et al., 1997; Niang et al., 2002). Even in the absence of external P inputs, annual maize yields were significantly higher in maize-legume fallow rotations than in continuous maize or maize-natural fallow rotations (Niang et al., 2002; Smestad et al., 2002), suggesting improved P availability.

Higher yields and related P exports will deplete available P which to a certain extent can be replenished from less soluble P pools, depending on the total P stock of the soil and its buffering capacity. With the regular inclusion of a one-season fallow into the crop rotation, depletion of available P may, however, also occur as a result of increased biological immobilization. After a single fallow of 7-17 months duration in western Kenya, no changes in inorganic P pools were detected, whereas increased levels of soil organic matter, especially its labile and microbial fractions, and associated contents of N...
Chapter 1: Maize productivity and nutrient dynamics in maize-fallow rotations

and P were observed (Maroko et al., 1998; 1999; Smestad et al., 2002). However, limited understanding of the factors regulating microbial activity, P mineralization rates and P cycling prevents a prediction how system productivity will be affected by improved fallows in the long term.

We compared plant production, N and P balances and soil properties in a field experiment with three crop rotations (continuous maize, maize-legume fallow and maize-natural fallow rotation) at two levels of P fertilization (0 and 50 kg P ha\(^{-1}\) yr\(^{-1}\), applied as TSP) over a period of 5.5 years in western Kenya. The legume species tested in this trial was *Crotalaria grahamiana* Wight & Arn. (subsequently referred to as crotalaria). Special attention was paid to transformations of soil P as the main limiting nutrient for plant growth by a) assessing available inorganic P b) determining stocks of inorganic and organic P in the top 0-50 cm and c) investigating factors limiting P uptake by the microbial biomass.

**Methods**

*Site and experimental design*

The field experiment was conducted between March 1997 and August 2002 in Central Kisa, Butere-Mumias District, western Kenya (0°09’ N, 34°33’ E) at an elevation of 1485 m. Mean annual rainfall as measured at this site from September 1997 to August 2002 was 1705 (SD ±197) mm. Monthly means above 150 mm which is the average potential evaporation per month (Jaetzold and Schmidt, 1982) occurred from March-May and October-November. The bimodal pattern results in two growing seasons: the long rainy season (LR) lasting from March to August and the (less reliable) short rainy season (SR) from September to February. The soil at this site is classified as a kaolinitic, isohyperthermic Kandiudalfic Eutrudox (USDA classification) or a Ferralsol (FAO) with 39% clay and 37% sand in the top 15 cm.

The experimental design was a randomized complete block with four replications. Three crop rotations (continuous maize (COM), maize-crotalaria fallow rotation (MCF) with *Crotalaria grahamiana* as improved fallow species and maize-natural fallow rotation (MNF)) were studied at two levels of P fertilization (0 and 50 kg P ha\(^{-1}\) yr\(^{-1}\)), applied as triple superphosphate (TSP) at the beginning of each LR season. At the same time, all plots received KCl applications of 100 kg K ha\(^{-1}\) yr\(^{-1}\). P and K fertilization was omitted
at the beginning of the last season reported here. Plot size was 6 m x 12 m during the first two seasons and 6 m x 6 m thereafter, because the plots were split before LR 1998 to study an additional P fertilization level which was not included in this study. At the beginning of each LR season, the soil was manually tilled down to 15 cm. Fertilizers were incorporated into the top 2 cm and maize was sown between mid-March and mid-April at 0.75 m x 0.25 m spacing. One to two months later, crotalaria was sown between the maize rows at the spacing of 0.75 m x 0.50 m. Maize was harvested from all plots between end of July and end of August. All maize residues were removed as is the common practice in order to minimize termite attraction to the field or to use the maize stover as fodder. Plots from the continuous maize treatment were then tilled and planted to maize between end of August and mid-September. Maize plots were harvested in January while fallows were cut in February or March. Crotalaria and weed biomass was determined and left within the plots to dry off so that crotalaria wood could be removed as fuelwood. Litterfall during the 6 weeks before cutting of the crotalaria fallows was determined by two 1.25 m² litter traps per plot in SR 1997 and 1998. Dominant species in the natural fallows were erect weeds such as *Guizotia scabra* (Vis) Chiov. and *Hibiscus cannabinus* L. growing in a dense soil cover of grasses and sedges (mainly *Digitaria* and *Cyperus* spp.) and herbs such as *Richardia scabra* L. In all crop rotations, the non-woody fallow and weed biomass was incorporated into the soil by manual hoeing down to 15 cm 2-5 weeks before maize was planted in the following LR season. Current Kenyan hybrid varieties were sown (H511 during LR 1997, H512 between SR 1997 and 1998, and H513 thereafter). Maize plots were weeded 1-3 times during each season as necessary. Weed biomass was recorded (data missing for LR 2000 and 2001) and returned to the plots. Weed biomass was lowest in COM during 2 out of 3 monitored LR seasons, indicating that the fallows did not reduce weed pressure in this trial. Populations of the parasitic weed striga (*Striga hermonthica*) were low (0.2±0.2 plants m⁻²) and striga counts were discontinued after LR 2000. Pesticides to control stalk borer and termites were generally applied once per season.

N and P concentrations in harvested maize were determined in all treatments on grain, rachis and stover separately (LR 1998, 1999 and 2000). At fallow harvest, subsamples from crotalaria leaves, pods, wood and litter (SR 1997 and 1998), and from the weed biomass in the natural fallow plots (SR 1998, 1999 and 2000) were taken for analysis.
To check for an effect of P fertilization on biological nitrogen fixation (BNF), leaf samples of crotalaria and *Guizotia scabra* collected in January 2000 from all MCF and MNF plots were ball-milled and analyzed for δ¹⁵N using a stable isotope mass spectrometer (Europa Scientific, Crewe, UK).

N and P concentration in maize grain, rachis and stover were affected by P fertilization, but not by crop rotations. Average N and P concentrations for −P and +P treatments, respectively, were therefore used to calculate maize N and P uptake. Concentrations differed between seasons but no significant interaction between P fertilization and season occurred. Where no season-specific determination was available, average concentrations of all measured seasons were used (Table 1.1). The same applied for N and P concentration in crotalaria and weed biomass. Mean N and P concentrations of weeds in the natural fallow plots were also used to calculate weed N and P uptake during the SR season in the other crop rotations. As δ¹⁵N values of -0.21 and 4.20 %o for crotalaria and *Guizotia scabra*, respectively, were in the same range as values determined by Gathumbi et al. (2002) under non-PK limiting conditions on a similar type of soil, their estimation of 75% of N in above-ground crotalaria biomass being derived from BNF was used for the calculation of N budgets.

Table 1.1: N and P concentrations in maize, crotalaria and weed biomass at harvest as affected by P fertilization

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<td>−P</td>
<td>+P</td>
<td>−P</td>
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<tr>
<td>maize</td>
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<tr>
<td>grain</td>
<td>11.4</td>
<td>10.8</td>
<td>1.6</td>
<td>1.9</td>
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<tr>
<td>stover</td>
<td>8.0</td>
<td>6.9</td>
<td>0.6</td>
<td>0.6</td>
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<tr>
<td>rachis</td>
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<td>7.0</td>
<td>0.6</td>
<td>0.7</td>
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<tr>
<td>crotalaria</td>
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<tr>
<td>leaves</td>
<td>23.8</td>
<td>25.6</td>
<td>1.2</td>
<td>1.5</td>
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<tr>
<td>pods</td>
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<tr>
<td>wood</td>
<td>9.9</td>
<td>10.8</td>
<td>0.4</td>
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<td>weeds</td>
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<td>11.8</td>
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* P = 0.01-0.05, ** P = 0.01-0.001, *** P < 0.001

Table 1.1: N and P concentrations in maize, crotalaria and weed biomass at harvest as affected by P fertilization

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<td>−P</td>
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<td>maize</td>
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<tr>
<td>grain</td>
<td>11.4</td>
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<tr>
<td>stover</td>
<td>8.0</td>
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<tr>
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<td>7.0</td>
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<td>0.6</td>
<td>0.7</td>
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<tr>
<td>crotalaria</td>
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<td>leaves</td>
<td>23.8</td>
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<td>pods</td>
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<td>wood</td>
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<td>weeds</td>
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<td>11.8</td>
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* Effect of crop rotation was not significant (ns), −P: 0, +P: 50 kg P ha⁻¹ yr⁻¹ applied as TSP

* Determined on weeds in MNF at the end of the SR season
Soil sampling

Composite soil samples from 15 random cores were collected from each plot (0-15 cm) before the establishment of the field experiment in March 1997 and in January 2000, 2001 and 2002 (at the end of the third, fourth and fifth fallow phase, respectively), when maize had been harvested and the fallows were still standing in the field. In 2001, the sampling was extended to the 15-30 cm and 30-50 cm layers. Field-moist samples were sieved at 4 mm to remove coarse plant debris and stored at 4°C. About 200 g of each sample were air-dried and sieved at 2 mm.

Field bulk density in the 0-15 cm layer was determined in November 2001 with cylinders of 5 cm diameter in all treatments (two samples per plot). No significant effect of crop rotation or P fertilization was found and the average bulk density of 1.00 (SD ±0.05) g cm⁻³ was used to calculate nutrient stocks. Bulk densities for the 15-30 and 30-50 cm layers were 1.03 (SD ±0.05) and 1.00 (SD ±0.02) g cm⁻³, respectively, as determined in four plots distributed over the experimental area. Roots were washed out from the 0-15 cm bulk density samples over a 1 mm sieve to obtain an estimate of root dry weight.

Analytical procedures

The following analyses were done on air-dried soils, following the procedures given by Anderson and Ingram (1993): pH_{H₂O} (soil:water-ratio 1:2.5), exchangeable acidity, calcium and magnesium by extraction with 1 M KCl, K and P extractable with 0.5 M NaHCO₃ + 0.01 M EDTA (modified Olsen, P_{Olsen}) and Bray-I-P by extraction with 0.03 M NH₄F + 0.025 M HCl (P_{Bray}). Following the method by Saunders and Williams (1955), P extracted with 0.5 M H₂SO₄ from non-ignited soils (P_{H₂SO₄}) was assumed to be a fraction of total inorganic P, whereas the increase in H₂SO₄-extractable P after ignition (550°C, 1h) was assumed to be organic P (P₀). Total P (P_{tot}) was determined by digestion with H₂O₂/H₂SO₄ as described by Anderson and Ingram (1993) which was verified to extract similar amounts of P from these soils as Na₂CO₃ fusion. The concentration of P in all extracts was determined colorimetrically (Murphy and Riley, 1962).

Isotopic exchange kinetics (Fardeau, 1996) were performed on the samples collected in 2000 to further characterize the availability of inorganic P. In this method, a known
amount of radioactivity is introduced with carrier-free $^{33}\text{PO}_4$ into an equilibrated soil-solution system and the decrease of radioactivity in the solution measured over 100 min. The application of this method to soils containing very little available P is described in Bühler et al. (2003), including the colorimetric determination of the P concentration in the soil solution (cp) with malachite green (Ohno and Zibilske, 1991). The ratio between the radioactivity remaining in the solution after one minute of exchange ($r_1$) and the total introduced radioactivity (R) is correlated to the P sorption capacity (Tran et al., 1988) and the parameter n represents the rate of disappearance of radioactivity from the solution for exchange times longer than one minute. The pool of free ions which are immediately plant available is approximated by the amount of P exchangeable within 1 min ($E_{\text{imin}}$) in mg kg$^{-1}$ calculated as

$$E_{\text{imin}} = 10 * \text{cp} * \frac{R}{r_1}$$

with the factor 10 resulting from the soil:solution ratio of 1:10.

Total C and N ($C_{\text{tot}}$, $N_{\text{tot}}$) were determined on ball-milled subsamples using a CN analyzer (Carlo Erba Instruments, NA 1500, Rodano-Milano, Italy). In addition, the proportion of labile C ($C_1$) was estimated on the samples taken in 2001, using the KMnO$_4$-oxidation method described by Blair et al. (1995) but with direct C determination in the soil residue as suggested by Shang and Tiessen (1997). $C_1$ was calculated as the difference between $C_{\text{tot}}$ and C content in the residue.

The remaining analyses were done on field-moist samples. Mineral N ($N_{\text{min}}$) was extracted with $2M$ KCl and the concentration of ammonium and nitrate determined colorimetrically. C and N held in the microbial biomass were determined by 24 h fumigation followed by extraction with 0.5 $M$ K$_2$SO$_4$ as described by Vance et al. (1987), with measurement of total C and N in the extracts using a Dimatoc 100 apparatus (Dimatec, Essen, Germany). P held in the microbial biomass was determined by simultaneous liquid fumigation and extraction with anion-exchange resin membranes (BDH #55164) in bicarbonate form for 16 h as described by Kouno et al. (1995), but using hexanol as the fumigant instead of chloroform which was found to dissolve the anion-exchange membranes. Hexanol has been shown to be as effective a fumigant as chloroform (McLaughlin et al., 1986). Subsamples with addition of an inorganic P spike equal to 5 mg P kg$^{-1}$ were included in order to account for sorption of released P during the extraction period. Microbial C, N and P are reported as amounts rendered
extractable by chloroform (C\textsubscript{chl} and N\textsubscript{chl}) and hexanol fumigation (P\textsubscript{hex}), respectively, without the use of a conversion factor. Amounts of P extracted from unfumigated subsamples are reported as resin-extractable P (P\textsubscript{resin}). Directly after the sampling in 2000, two samples per plot of 40 g dry matter each were incubated at a water content of 300 g kg\textsuperscript{-1} dry matter at 25°C for 30 days and soil respiration was determined weekly by trapping CO\textsubscript{2} in 0.5 M NaOH followed by titration with 0.15 M HCl (Alef, 1995). The metabolic quotient (qCO\textsubscript{2}), i.e. the ratio between soil respiration and microbial C, was calculated with values determined during the last week of incubation when respiration rates had stabilized.

**Incubation experiment**

In addition to the characterization of the field soils, the effect of C and N availability on microbial P was studied in an incubation experiment with additions of glucose and NH\textsubscript{4}NO\textsubscript{3}. Composite samples which had been collected in 2000 from the treatments MCF±P and stored field-moist at 4°C were pre-incubated at a water content of 250 g kg\textsuperscript{-1} (60\% water holding capacity) for 10 days at 25°C in the dark. Three levels of C and N additions were chosen: a) 0.5 mg C without N, b) 2.5 mg C without N and c) 2.5 mg C with 0.25 mg N g\textsuperscript{-1} soil. Non-amended and sole-N amended controls were also included. Samples were incubated at 25°C and analyzed after 2, 4, 7 and 14 days. Treatment effects on P\textsubscript{hex} and P\textsubscript{resin} are presented as the difference between treatments and the non-amended controls.

**Statistical analysis**

Statistical analysis was carried out with SYSTAT (SPSS 2000). Maize yield during LR seasons, cumulative yield of 5.5 years, recycled biomass and soil properties were tested by three-way ANOVA, with the factors crop rotation, P fertilization, field block, and the crop rotation x P fertilization interaction. Maize yield during SR seasons was analyzed by two-way ANOVA with the factors P fertilization and field block. Soil analyses were done with 1-3 analytical replicates per sample. The statistical analysis, however, was performed with one mean value per plot. Fractions (rI/R, Al saturation) and quotients (qCO\textsubscript{2}) log-transformed before the statistical analysis. Multiple comparisons using Tukey’s test were done whenever the ANOVA indicated significant differences ($P \leq 0.05$).
Results and Discussion

Fallow productivity

During the first SR season (1997), crotalaria growth was not significantly affected by P fertilization (Figure 1.1). The recycled biomass in MCF of 5.3 Mg ha\(^{-1}\) (pods, leaves, and weeds) and associated N and P (96 and 8 kg ha\(^{-1}\)) were somewhat lower than those observed by Smestad et al. (2002) for a crotalaria fallow in a similar trial in western Kenya (6.4 Mg DM, 163 kg N and 11 kg P ha\(^{-1}\), including litterfall). During the following SR seasons, crotalaria growth decreased dramatically (Figure 1.1). For 1998, this may have been due to the exceptionally low rainfall in the second half of the year. The continuing decrease in crotalaria performance, however, must be attributed to pest problems. Infestation of crotalaria fallow with the plant hopper *Hilda patruelis* and resulting damage including the colonization of stem lesions by saprophytic fungi were observed to increase in western Kenya where improved fallows were planted (Girma, 2002). According to Desaeger (2001), crotalaria fallows also lead to a build-up of populations of root-lesion nematodes (*Pratylenchus* spp.), some of which might have weakened crotalaria plants as well.

![Figure 1.1: Above-ground biomass of *Crotalaria grahamiana* ± P fertilization during 5 short rainy seasons in western Kenya (error bars show the standard deviation of the standing biomass at fallow harvest; no litter data available for 1999-2001)](image-url)
Table 1.2: Non-woody biomass recycled during and at the end of 5 short rainy seasons and sum of associated N and P

<table>
<thead>
<tr>
<th>Crop rotation</th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>sum</th>
<th>sum</th>
<th>sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM(^b)</td>
<td>1.5</td>
<td>0.9</td>
<td>0.4</td>
<td>1.1</td>
<td>1.0</td>
<td>4.8</td>
<td>56</td>
<td>6.8</td>
</tr>
<tr>
<td>MCF</td>
<td>5.3</td>
<td>2.6</td>
<td>3.6</td>
<td>3.8</td>
<td>3.3</td>
<td>18.6</td>
<td>275</td>
<td>26.8</td>
</tr>
<tr>
<td>MNF</td>
<td>3.2</td>
<td>2.4</td>
<td>4.4</td>
<td>5.5</td>
<td>2.6</td>
<td>18.1</td>
<td>207</td>
<td>25.6</td>
</tr>
<tr>
<td>SED(^d)</td>
<td>0.5</td>
<td>0.3</td>
<td>0.5</td>
<td>0.6</td>
<td>0.3</td>
<td>1.1</td>
<td>14</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P fertilization</th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>sum</th>
<th>sum</th>
<th>sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>-P(^c)</td>
<td>3.6</td>
<td>1.8</td>
<td>2.7</td>
<td>3.6</td>
<td>2.4</td>
<td>14.0</td>
<td>179</td>
<td>17.3</td>
</tr>
<tr>
<td>+P</td>
<td>3.0</td>
<td>2.2</td>
<td>2.9</td>
<td>3.3</td>
<td>2.2</td>
<td>13.7</td>
<td>180</td>
<td>22.2</td>
</tr>
<tr>
<td>SED</td>
<td>0.4</td>
<td>0.2</td>
<td>0.6</td>
<td>0.5</td>
<td>0.3</td>
<td>0.9</td>
<td>11</td>
<td>1.3</td>
</tr>
</tbody>
</table>

\(^a\) sum of weeds, crotalaria leaves and pods (no litter data included)
\(^b\) COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation
\(^c\) Within columns, means followed by the same letter are not significantly different (\(P = 0.05\)) by Tukey’s multiple range test; ns: not significant; interactions between crop rotation and P fertilization were not significant
\(^d\) SED: standard error of the difference in means
\(^e\) -P: 0, +P: 50 kg P ha\(^{-1}\) yr\(^{-1}\) applied as TSP
The biomass production of the crotalaria fallow was superior to that of the natural fallow only during the first SR season of the trial (Table 1.2). Likewise, the sum of biomass and associated P (18 Mg and 26 kg ha\(^{-1}\)) recycled during 5 SR seasons did not differ between MCF and MNF, but the cumulative amount of recycled N was 70 kg ha\(^{-1}\) higher in MCF than in MNF. Compared to the weeds in COM, both fallows types produced an additional 1.5-4 Mg ha\(^{-1}\) recyclable dry matter during each of the SR seasons. In the trial reported by Smestad et al. (2002), the recycled biomass in a one-season natural fallow did not differ from the mass of weeds in continuous maize. However, their results agree with our finding that P fertilization did not affect the amount of recycled biomass and associated N for any of the crop rotations. As the soils in maize-fallow rotations were tilled only at the beginning of each LR season, applied P fertilizer was located in the top 2 cm during the first SR season (1997) and may not have been available to fallows. During the following SR seasons when previously applied fertilizer had been incorporated, crotalaria and weed growth was apparently limited by other factors than P availability. In crotalaria leaves sampled in January 2000, also no effect of P fertilization on BNF was found, as \(\delta^{15}N\) values of -0.21 (SD ±0.50) \(\%\) showed no significant difference between -P and +P treatments. P fertilization did, however, increase the cumulative amount of P recycled during 5 fallow seasons by 28% due to its effect on P concentration in crotalaria leaves and weeds (Tables 1.1, 1.2).

**Maize yield**

During the LR season 1997 when maize was grown in all crop rotations, grain yield was significantly affected only by P fertilization (Table 1.3). Thus, maize yield was not decreased by the interplanted crotalaria fallow, in contrast to the observations by Smestad et al. (2002). P fertilization increased yields during each LR season but not during any of the SR seasons. Presumably, the potential yield under water-limited conditions was too small to detect a response to fertilization, similar to the conclusion by Reid et al. (2002) from the calibration of a crop model to maize yields across a wide range of conditions. A significant effect of the crop rotations on maize yields was observed in the LR seasons 1998, 1999 and 2001, with the crop rotations ranging in the order MCF>MNF=COM. Concentrations of N and P in maize grain, stover and rachis
Table 1.3: Effect of crop rotation and P fertilization on seasonal and cumulative maize grain yield during 5.5 years in western Kenya

| treatment<sup>b</sup>   | df | LR97<sup>a</sup> | SR97 | LR98 | SR98 | LR99 | SR99 | LR00 | SR00 | LR01 | SR01 | LR02 | sum  |
|-------------------------|----|------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| COM-P                   | 1.3 b<sup>c</sup> | 0.1 ns | 1.2 c | 0.1 ns | 0.9 c | 1.5 ns | 0.8 b | 0.9 ns | 0.5 b | 0.3 ns | 0.1 b | 7.8 c |
| MCF-P                   | 1.8 ab | 3.2 b | 1.9 bc | 2.1 ab | 0.7 b | 0.4 b | 10.0 bc |
| MNF-P                   | 1.2 b | 2.0 bc | 1.3 c | 1.8 ab | 0.5 b | 0.4 b | 7.2 c |
| COM+P                   | 2.1 ab | 0.2 ns | 2.8 bc | 0.5 ns | 3.6 b | 2.4 ns | 2.6 ab | 1.3 ns | 0.6 b | 1.0 ns | 0.7 ab | 17.7 ab |
| MCF+P                   | 2.0 ab | 5.5 a | 5.8 a | 3.6 a | 2.7 a | 2.3 a | 21.9 a |
| MNF+P                   | 2.5 a | 3.6 a | 2.4 bc | 2.6 ab | 1.1 b | 1.1 ab | 13.3 bc |
| SED<sup>d</sup>         | 0.3 | 0.03 | 0.6 | 0.1 | 0.6 | 0.6 | 0.2 | 0.4 | 0.3 | 0.5 | 2.4 |

Source of variation

|                      | df | LR97<sup>a</sup> | SR97 | LR98 | SR98 | LR99 | SR99 | LR00 | SR00 | LR01 | SR01 | LR02 | sum  |
|----------------------|----|------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| Crop rotation (R)    | 2  | ns               | ***  | ***  | ns   | **   | ns   |      |      |      |      |      |      |      |
| P fertilization (P)  | 1  | ***  | ns   | ***  | ns   | ***  | ns   | ***  | ns   | ***  | ns   | **   | ***  |
| R x P                | 2  | ns               | ns   |      |      |      |      |      |      |      |      |      |      |      |

---

<sup>a</sup> P = 0.01-0.05, ** P = 0.01-0.001, *** P < 0.001, ns: not significant
<sup>b</sup> LR: long rainy season, SR: short rainy season
<sup>c</sup> COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation, -P: 0, +P: 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> applied as TSP
<sup>d</sup> Within columns, means followed by the same letter are not significantly different (P = 0.05) by Tukey's multiple range test
<sup>e</sup> SED: standard error of the difference in means

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Chapter 1: Maize productivity and nutrient dynamics in maize-fallow rotations

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were not significantly affected by crop rotations and only partly by P fertilization (Table 1.1). As observed by Smestad et al. (2002), any increase in nutrient availability in the soil was used by the plant to produce more biomass rather than to increase nutrient concentrations.

The amount of above-ground fallow and weed biomass incorporated into the soil explained 61 and 64% of the yield variation in the first post-fallow season (LR 1998) for -P and +P treatments, respectively. In the further course of the trial, maize yield was significantly related to the amount of biomass incorporated into the soil at the beginning of the season only in LR 2000 for -P and LR 2002 for +P treatments, respectively. With the additional inclusion of cumulative biomass inputs from previous fallow seasons into the regression, the model was improved in all seasons and the relationship became significant also for LR 2001 (-P and +P) and 2002 (-P), suggesting a residual effect of previous fallows.

Significant interactions between crop rotation and P fertilization occurred in two LR seasons (Table 1.3), when maize yield was significantly higher in MCF than in the other two crop rotations in the presence of P fertilization only. Possibly, the additional N inputs by the crotalaria fallow could be fully exploited only after P limitation had been removed. Alternatively, nematodes may be most deleterious when plant growth is strongly limited by P availability. Pratylenchus spp. are important pathogenic nematodes on maize in Kenya, and Desaeger (2001) observed a doubling of maize yield by nematicide applications in the absence of inorganic fertilizer compared to 25 and 12% yield increase at medium and high NPK fertilizer application rates, respectively.

After 5 seasons, the cumulative grain yield was significantly higher in MCF than in COM by 3.1 and 4.2 Mg ha$^{-1}$ for -P and +P treatments, respectively, suggesting that the maize-crotalaria fallow rotation has the potential to increase yields beyond the compensation for the yield forgone during the fallow phase. After 11 seasons, however, the cumulative yield increase in MCF compared to COM was no longer significant due to comparatively high maize yields obtained during two SR seasons (1999 and 2000) in COM (Table 1.3). P fertilization approximately doubled the cumulative yield irrespective of the crop rotation. More than a compensation of lost yields was not achieved by improved fallows in Rwanda (Drechsel et al., 1996), whereas a significant response of maize to additions of inorganic P as low as 10-15 kg P ha$^{-1}$ has frequently been observed in western Kenya (Jama et al., 1997; Nziguheba et al., 2000).
Table 1.4: Range of annual N and P input and output (minimum and maximum values) and total N and P balance as affected by crop rotation and P fertilization during 5.5 years in western Kenya

<table>
<thead>
<tr>
<th>treatment</th>
<th>annual N</th>
<th>annual P</th>
<th>total balance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>input kg ha(^{-1}) yr(^{-1})</td>
<td>output kg ha(^{-1}) yr(^{-1})</td>
<td>input kg ha(^{-1})</td>
</tr>
<tr>
<td>COM-P</td>
<td>0</td>
<td>16.9-60.5</td>
<td>0</td>
</tr>
<tr>
<td>MCF-P</td>
<td>0.4-62.6</td>
<td>11.0-92.0</td>
<td>0</td>
</tr>
<tr>
<td>MNF-P</td>
<td>0</td>
<td>10.8-42.7</td>
<td>0</td>
</tr>
<tr>
<td>COM+P</td>
<td>0</td>
<td>35.4-96.0</td>
<td>50</td>
</tr>
<tr>
<td>MCF+P</td>
<td>0.7-51.1</td>
<td>47.3-133.2</td>
<td>50</td>
</tr>
<tr>
<td>MNF+P</td>
<td>0</td>
<td>20.7-68.3</td>
<td>50</td>
</tr>
</tbody>
</table>

\(^{a}\) Estimated assuming that 75% of N in above-ground crotalaria biomass is derived from BNF (Gathumbi et al., 2002)

\(^{b}\) COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation, \(-P\): 0, \(+P\): 50 kg P ha\(^{-1}\) yr\(^{-1}\) applied as TSP

\(^{c}\) Within columns, means followed by the same letter are not significantly different (\(P = 0.05\)) by Tukey’s multiple range test

\(^{d}\) SED: standard error of the difference in means

**N and P balance**

Annual N and P balances varied by a factor of 2-4 (Table 1.4). According to the cumulative balances after 5.5 years, 75-85% of the applied 250 kg P ha\(^{-1}\) had not been exported. On the other hand, the negative N balance observed in the \(-P\) treatments was aggravated through P fertilization by 90-195 kg N ha\(^{-1}\), depending on the crop rotation. Without P fertilization, N inputs from BNF slightly improved the N balance in MCF compared to COM, whereas with P fertilization, they merely compensated the additional N exported with higher maize and fuelwood yields.

The mean annual export rates in COM–P of 43 kg N and 4 kg P ha\(^{-1}\) were close to the depletion rates of 36 kg N and 5 kg P ha\(^{-1}\) estimated by Smaling et al. (1997) for the east African highlands. Maize residues made up 62 and 48% of N and P exports, respectively, which could thus be reduced if nutrients were returned in the form of animal manure. Removal of crotalaria wood constituted an export of 1 kg P ha\(^{-1}\) during the first fallow season and the resulting increase in maize yield in LR 1998 an additional export of 5 kg P ha\(^{-1}\) from MCF–P compared to COM–P.
Unless subsidized, annual fertilizer applications of 50 kg P ha\(^{-1}\) are not affordable to most small-scale farmers in western Kenya. However, even smaller amounts of P can effectively increase yields. To balance the outputs, the maize-crotalaria fallow rotation would require annual additions in the order of 20 kg P ha\(^{-1}\) and 70 kg N ha\(^{-1}\) if N inputs from BNF amount to about 60 kg ha\(^{-1}\).

**C, N and P content in the 0-15 cm soil layer**

In the samples taken in 1997 before the establishment of the trial, there were no significant differences in levels of available inorganic P (\(P_{\text{ Olsen}}\), \(P_{\text{ resin}}\)), K as well as C\(_{\text{tot}}\) and N\(_{\text{tot}}\) between plots later subjected to the different crop rotations and P fertilization levels (data not shown). Treatment effects on the various soil properties were generally similar for the samples taken in 2000, 2001 and 2002, respectively, and except for increasing levels of inorganic P in +P treatments, no time trends were observed. Unless stated otherwise, the results from 2000 (end of third fallow phase) were selected for presentation.

**Table 1.5: Soil pH, exchangeable acidity and cations in 0-15 cm depth as affected by crop rotation and P fertilization**

<table>
<thead>
<tr>
<th>Crop rotation</th>
<th>(\text{pH}_{\text{H}_2\text{O}})</th>
<th>(\text{EA}^{a})</th>
<th>(\text{Ca}_{\text{ex}}^{a})</th>
<th>(\text{Mg}_{\text{ex}}^{a})</th>
<th>(\text{K}_{\text{ex}}^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM(^b)</td>
<td>4.9 b(^c)</td>
<td>1.9 ns</td>
<td>2.8 ns</td>
<td>0.9 b</td>
<td>0.12 ns</td>
</tr>
<tr>
<td>MCF</td>
<td>5.0 ab</td>
<td>1.4 ns</td>
<td>3.4 ns</td>
<td>1.0 ab</td>
<td>0.09 ns</td>
</tr>
<tr>
<td>MNF</td>
<td>5.1 a</td>
<td>1.4 ns</td>
<td>3.2 ns</td>
<td>1.2 a</td>
<td>0.10 ns</td>
</tr>
<tr>
<td>SED(^d)</td>
<td>0.06</td>
<td>0.2</td>
<td>0.4</td>
<td>0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>P fertilization</th>
<th>(\text{Ca}_{\text{ex}}^{a})</th>
<th>(\text{Mg}_{\text{ex}}^{a})</th>
<th>(\text{K}_{\text{ex}}^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>–P(^e)</td>
<td>5.0 ns</td>
<td>1.5 ns</td>
<td>3.2 ns</td>
</tr>
<tr>
<td>+P</td>
<td>4.9 ns</td>
<td>1.6 ns</td>
<td>3.1 ns</td>
</tr>
<tr>
<td>SED</td>
<td>0.05</td>
<td>0.2</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\(^a\) EA, \(\text{Ca}_{\text{ex}}\), \(\text{Mg}_{\text{ex}}\), \(\text{K}_{\text{ex}}\): exchangeable acidity, Ca, Mg and K, respectively

\(^b\) COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation

\(^c\) Within columns, means followed by the same letter are not significantly different (\(P = 0.05\)) by Tukey’s multiple range test; ns: not significant.

Interactions between crop rotation and P fertilization were not significant.

\(^d\) SED: standard error of the difference in means

\(^e\) –P: 0, +P: 50 kg P ha\(^{-1}\) yr\(^{-1}\) applied as TSP
Table 1.6: Inorganic P and N availability in 0-15 cm depth as affected by crop rotation and P fertilization

<table>
<thead>
<tr>
<th></th>
<th>ep mg l⁻¹</th>
<th>r1/R</th>
<th>n</th>
<th>E₁min</th>
<th>P₉₁₀</th>
<th>P₀₂₅</th>
<th>P₁₀</th>
<th>Ν₉₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crop rotation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COM</td>
<td>0.002 ns c</td>
<td>0.019 ns</td>
<td>0.50 ns</td>
<td>0.8 ns</td>
<td>4.3 ns</td>
<td>3.0 ns</td>
<td>8.5 ns</td>
<td>15.5 b</td>
</tr>
<tr>
<td>MCF</td>
<td>0.002 ns</td>
<td>0.020 ns</td>
<td>0.50 ns</td>
<td>0.8 ns</td>
<td>4.0 ns</td>
<td>2.5 ns</td>
<td>7.7 ns</td>
<td>21.9 a</td>
</tr>
<tr>
<td>MNF</td>
<td>0.002 ns</td>
<td>0.019 ns</td>
<td>0.49 ns</td>
<td>0.9 ns</td>
<td>4.2 ns</td>
<td>2.6 ns</td>
<td>7.8 ns</td>
<td>17.3 b</td>
</tr>
<tr>
<td>SED</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.007</td>
<td>0.12</td>
<td>0.6</td>
<td>0.4</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>P fertilization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-P</td>
<td>0.001 b</td>
<td>0.018 ns</td>
<td>0.52 a</td>
<td>0.7 b</td>
<td>1.7 b</td>
<td>1.3 b</td>
<td>4.9 b</td>
<td>18.0 ns</td>
</tr>
<tr>
<td>+P</td>
<td>0.002 a</td>
<td>0.020 ns</td>
<td>0.48 b</td>
<td>1.0 a</td>
<td>6.6 a</td>
<td>4.1 a</td>
<td>11.1 a</td>
<td>18.5 ns</td>
</tr>
<tr>
<td>SED</td>
<td>0.0001</td>
<td>0.002</td>
<td>0.006</td>
<td>0.10</td>
<td>0.5</td>
<td>0.3</td>
<td>0.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a N₉₁₀: sum of ammonium and nitrate
b COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation
c Within columns, means followed by the same letter are not significantly different (P = 0.05) by Tukey’s multiple range test; ns: not significant. Interactions between crop rotation and P fertilization were not significant except in the case of n (significant interaction between MNF and P fertilization).
d SED: standard error of the difference in means
e -P: 0, +P: 50 kg P ha⁻¹ yr⁻¹ applied as TSP
f lower levels of $P_{Bray}$ (2.4 and 11.1) and $N_{min}$ (10.5 and 10.6, for -P and +P treatments, respectively) with similar cropping effects observed in 2001.
Soil pH$_{\text{H}_2\text{O}}$ and extractable cations showed few significant effects of crop rotation and P fertilization (Table 1.5). COM was always lowest in pH and highest in exchangeable acidity, about 88% of which is represented by Al in soils of western Kenya (Smithson, unpublished). The relatively low effective cation exchange capacity of 5.8 cmol$_e$ kg$^{-1}$ did not differ between crop rotations, but base saturation was lower in COM than in MCF and MNF. However, Al saturation never exceeded 35%, indicating that Al toxicity was not a major constraint to maize growth (Smithson and Sanchez, 2001).

Without P fertilization, $P_{\text{resin}}$, $P_{\text{Olsen}}$ and $P_{\text{Bray}}$ were below 5 mg P kg$^{-1}$ (Table 1.6). Likewise, very low concentrations of P in the soil solution (cp), values for the parameters $r_1/R$ and $n$ of the isotopic exchange kinetics of 0.02 and 0.5, respectively, and resulting amounts of P exchangeable within one minute ($E_{1\text{min}}$) of less than 1 mg P kg$^{-1}$ indicate that this soil is very low in available P and has a high P sorption capacity (Tran et al., 1988). Other studies have classified similar soils in western Kenya as moderately P sorbing (~310 mg P kg$^{-1}$ soil at a soil solution P concentration of 0.2 mg P l$^{-1}$) according to P sorption isotherms (Nziguheba et al., 1998; Smestad et al., 2002).

Crop rotations did not affect levels of available P, whereas $N_{\text{min}}$ contents were significantly higher in MCF than in the other two crop rotations. Likewise, the systems generally ranged in the order MCF>MNF>COM with regard to $C_{\text{tot}}$, $N_{\text{tot}}$ and $P_o$ in the topsoil, whereas P fertilization did not affect any of these properties (Table 1.7). Higher levels of soil organic matter in MCF and MNF than COM are probably responsible for the observed differences in pH as well as base saturation (Haynes and Mokolobate, 2001).

The ignition method has been shown to overestimate $P_o$ in highly weathered soils due to changes in acid solubility of inorganic P during ignition (Condron et al., 1990). Nevertheless, it is valid for the estimation of differences between treatments on the same soil type. The increase in $P_o$ in MCF compared to COM of 22 mg P kg$^{-1}$ at the end of the third fallow phase compares well with the observed increase in NaOH-extractable $P_o$ of 14 mg P kg$^{-1}$ after 17 months of sesbania fallow (Maroko et al., 1999) and 16 mg P kg$^{-1}$ after 7 months of crotalaria fallow (Smestad et al., 2002) in western Kenya. Similar to our results, Smestad et al. (2002) did not find a difference in $P_o$ accumulation between -P and +P treatments. Higher apparent transformation of fertilizer P into
Table 1.7: Organic and microbial soil characteristics in 0-15 cm depth as affected by crop rotation and P fertilization

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;tot&lt;/sub&gt;</th>
<th>C&lt;sub&gt;i&lt;/sub&gt;</th>
<th>N&lt;sub&gt;tot&lt;/sub&gt;</th>
<th>P&lt;sub&gt;O&lt;/sub&gt;</th>
<th>C&lt;sub&gt;chl&lt;/sub&gt;</th>
<th>N&lt;sub&gt;chl&lt;/sub&gt;</th>
<th>P&lt;sub&gt;hex&lt;/sub&gt;</th>
<th>cumul. respiration</th>
<th>daily respiration</th>
<th>qCO&lt;sub&gt;2&lt;/sub&gt;</th>
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<tr>
<td></td>
<td>g kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>mg kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>mg C kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>mg C kg&lt;sup&gt;-1&lt;/sup&gt; d&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>µg C mg&lt;sup&gt;-1&lt;/sup&gt; C&lt;sub&gt;chl&lt;/sub&gt; h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
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<tr>
<td><strong>Crop rotation</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.6 b</td>
<td>9.5 c</td>
<td>1.6 b</td>
<td>264 b</td>
<td>98 b</td>
<td>13.3 b</td>
<td>3.5 b</td>
<td>139 b</td>
<td>3.3 b</td>
<td>1.4 b</td>
</tr>
<tr>
<td>MCF</td>
<td>26.8 a</td>
<td>13.2 a</td>
<td>2.0 a</td>
<td>286 a</td>
<td>147 a</td>
<td>21.7 a</td>
<td>6.4 a</td>
<td>248 a</td>
<td>5.4 a</td>
<td>1.5 b</td>
</tr>
<tr>
<td>MNF</td>
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<td>11.5 b</td>
<td>1.8 ab</td>
<td>272 ab</td>
<td>128 ab</td>
<td>19.4 a</td>
<td>5.3 a</td>
<td>273 a</td>
<td>6.6 a</td>
<td>2.2 a</td>
</tr>
<tr>
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<td>0.6</td>
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<td>8</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−P&lt;sup&gt;f&lt;/sup&gt;</td>
<td>25.1 ns</td>
<td>11.3 ns</td>
<td>1.8 ns</td>
<td>273 ns</td>
<td>124 ns</td>
<td>17.3 ns</td>
<td>4.9 ns</td>
<td>214 ns</td>
<td>4.9 ns</td>
<td>1.6 ns</td>
</tr>
<tr>
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<td>275 ns</td>
<td>124 ns</td>
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</tr>
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<td>0.06</td>
<td>6</td>
<td>7</td>
<td>1.2</td>
<td>0.4</td>
<td>13</td>
<td>0.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> C<sub>i</sub>: labile C (removed from samples taken in 2001 by oxidation with 0.333 M KMnO<sub>4</sub>)

<sup>b</sup> Cumulative CO<sub>2</sub>-release during 30 days of incubation, daily respiration rate during the last week and resulting qCO<sub>2</sub>, respectively

<sup>c</sup> COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation

<sup>d</sup> Within columns, means followed by the same letter are not significantly different (P = 0.05) by Tukey’s multiple range test; ns: not significant.

Interactions between crop rotation and P fertilization were not significant.

<sup>e</sup> SED: standard error of the difference in means

<sup>f</sup> −P: 0, +P: 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> applied as TSP
organic P was observed under grass-legume pasture than under continuous rice in an Oxisol in Colombia (Oberson et al., 2001). Soil under grass-legume pasture in another experiment in Colombia had higher levels of inorganic P fractions ($P_{\text{resin}}$, NaHCO$_3$-P$_i$ and NaOH-P$_i$) as well as NaOH-P$_o$ than under grass-only pasture although P inputs had been similar in both systems (Oberson et al., 1999). In addition, P sorption as indicated by values of r1/R and the amount of P required to raise the soil solution concentration to 0.2 mg P l$^{-1}$ was significantly lower in the grass-legume pasture. This improvement in P availability was attributed to higher biological activity in the presence of legumes. In contrast, the legume fallow in our study increased levels of organic and microbial C, N and P without affecting available P and P sorption as indicated by values of r1/R or the recovery of the P spike included in the determination of microbial P (data not shown). This absence of an observable effect on P sorption could be related to the quality of recycled plant biomass which, for instance, had low nutrient concentrations except for N in crotalaria leaves and pods (Table 1.1). Accumulated P$_o$, however, can contribute to available P if it is mineralized. In the study presented by Maroko et al. (1999), the elevated levels of NaOH-P$_o$ as well as P contained in macro-organic matter a month after incorporation of fallow biomass had significantly decreased after three seasons of maize cropping, suggesting that P mineralization had occurred.

**Time trends in soil C and N**

Compared to the levels in 1997, average amounts of $C_{\text{tot}}$ in the topsoil had increased by 1.6-2.2 and 2.5-4.1 Mg C ha$^{-1}$ for MNF and MCF, respectively, whereas losses of 1.2-2.2 Mg C ha$^{-1}$ were observed after 4-6 years of continuous maize cropping (Figure 1.2). In the fallow systems, no further buildup of soil organic matter after the sampling in 2000 was recognizable. Possibly, a new equilibrium between biomass inputs and decomposition rate had been reached. In the tropics, this has been shown to happen within a few years (Feller and Beare, 1997).

Together with C, the total amount of N in the top 15 cm at the end of the third fallow phase (sampling 2000) had increased over pre-treatment levels (1997) by 315 and 210 kg ha$^{-1}$ for MCF and MNF, respectively, in spite of negative input-output balances (Table 1.4). For MCF, this increase is more than the 100 kg N ha$^{-1}$ recycled in crotalaria leaves and pods until this sampling, 75% of which may have been derived from biological nitrogen fixation. For MNF, an increase in topsoil N has to be attributed
either to non-symbiotic N$_2$ fixation or transfer of N from below the sampled 0-15 cm layer to the topsoil, as legumes were absent from the natural fallow except for an occasional self-sown crotalaria plant. Likewise, P uptake from below 15 cm depth could contribute to the observed increase of organic P in the topsoil compared to COM of 33 and 13 kg P ha$^{-1}$ for MCF and MNF, respectively, which was not accompanied by a change in available inorganic P. If soil layers below the top 15 cm significantly contribute to plant nutrient uptake, they should be included in estimates of nutrient stocks available for the systems against which nutrient outputs and recycling are viewed.

![Figure 1.2: Changes in concentration of C$_{tot}$ in the top 15 cm compared to pre-treatment values (1997) as influenced by the crop rotations (effect of P fertilization was not significant; box plots show the 25th and 75th percentiles and the median)](image)

**N and P content in the 15-50 cm layer**

The extended sampling down to 50 cm depth at the end of the fourth fallow phase showed that N$_{tot}$ was not significantly affected by the crop rotations below 0-15 cm (Figure 1.3). Thus, uptake of N from below 0-15 cm could not be shown against the large stock of total N of about 8000 kg ha$^{-1}$ in the top 50 cm. Throughout the profile, P$_{resin}$ was also not affected by the crop rotations. The non-significant trend of lower P$_{H_2SO_4}$ in MCF and MNF than in COM in the topsoil became significant in the 15-30 cm layer ($P = 0.001$) and was almost significant in the 30-50 cm layer ($P = 0.069$). As a
Chapter 1: Maize productivity and nutrient dynamics in maize-fallow rotations

Figure 1.3: Selected inorganic and organic soil properties in the top 50 cm as affected by crop rotation (COM: continuous maize, MCF: maize-crotalaria fallow rotation, MNF: maize-natural fallow rotation) and P fertilization (–P: 0, +P: 50 kg P ha\(^{-1}\) yr\(^{-1}\)) in western Kenya (soil layers followed by the same letter are not significantly different (P = 0.05) by Tukey’s multiple range test; bars show the standard error of the difference in means within a soil layer)
result, the total stock of $P_{\text{H}2\text{SO}_4}$ in the top 50 cm averaged over the two levels of P fertilization decreased significantly compared to COM by 38 and 35 kg P ha$^{-1}$ in MCF and MNF, respectively. It thus appears that both fallow types depleted $P_{\text{H}2\text{SO}_4}$ throughout the top 50 cm and returned the P to the soil with their recycled biomass. This transformation of inorganic into organic P amounted to about 1% of the total P stock of 3400 kg ha$^{-1}$ for the top 50 cm of unfertilized soil.

P fertilization increased $P_{\text{resin}}$ and $P_{\text{H}2\text{SO}_4}$ down to 50 cm, whereas $P_{\text{tot}}$ was not significantly affected below the top 15 cm due to high spatial variation. Compared to the $-P$ plots, $+P$ plots contained an additional 48, 26 and 38 kg $P_{\text{H}2\text{SO}_4}$ ha$^{-1}$ in the 15-50 cm layer of COM, MCF and MNF, respectively. This equals a fertilizer transfer below 15 cm of 13-24% of the 200 kg P ha$^{-1}$ applied until this sampling, some of which may have been caused by accidental deeper tillage. Averaged over crop rotations, $+P$ treatments contained an additional 140 kg $P_{\text{H}2\text{SO}_4}$ ha$^{-1}$ in the 0-50 cm layer, equal to 70% recovery of applied fertilizer P in $P_{\text{H}2\text{SO}_4}$. The greater depletion of $P_{\text{H}2\text{SO}_4}$ under fallows than continuous maize in both $-P$ and $+P$ treatments indicates that at least the 0-30 cm layer should be considered when assessing the P stocks available to fallows.

Factors affecting microbial biomass in the field

Compared to COM, levels of microbial C, N and P were increased by a factor of 1.5-1.8 in MCF and by a factor of 1.3-1.5 in MNF (Table 1.7). No effect of P fertilization on nutrients in the microbial biomass or soil respiration was detected. Soil respiration was also higher in both fallow systems than in COM, but while $C_{\text{tot}}$ and $C_{\text{chl}}$ were consistently lower in MNF than in MCF, soil respiration showed a reverse trend. This resulted in a significantly higher $q_{\text{CO}_2}$ for MNF than for MCF. However, the amount of labile C as indicated by KMnO$_4$-oxidation was lower in MNF than in MCF (Table 1.7) and the amount of non-labile C was similar for all soils. The high $q_{\text{CO}_2}$ in MNF may indicate a lower energetic efficiency of the microbial biomass than in MCF which can be caused by differences in soil organic matter quality, nutrient availability and/or the composition of the microbial biomass (Anderson and Domsch, 1990).

In our dataset, levels of soil organic C explain 53-73% of the variation in microbial C and P, depending on the sampling year. The additional inclusion of $N_{\text{min}}$ improves the model by up to 20 and 11% for $C_{\text{chl}}$ and $P_{\text{hex}}$, respectively. The inclusion of $P_{\text{resin}}$ on the other hand is not effective in improving the model except for $P_{\text{hex}}$ at the last sampling
Similarly, the quantity of microbial P in fertilized Oxisols in Colombia was not determined by available inorganic P (Oberson et al., 2001). Our data show that independent of soil P availability, the availability of C substrate constitutes the main condition for microbial biomass formation, at least when other environmental factors such as pH and soil water content are similar.

The close relationship between soil C and $P_{\text{hex}}$ leads to the conclusion that elevated levels of microbial P in the fallow systems down to 30 cm soil depth (Figure 1.3) must have been caused by residues incorporated below 15 cm through accidental tillage or by root inputs, including root exudates. Also $C_{\text{ahl}}$ and $N_{\text{ahl}}$ were significantly higher in the 15-30 cm layer in MCF and MNF compared to COM, whereas $C_{\text{tot}}$ was similar between all three systems (data not shown) and thus less sensitive to C inputs. Greater root-derived C inputs under fallows than under maize during the SR season may also have contributed to higher levels of microbial biomass observed in the top 15 cm, in addition to the effect of previous fallow biomass incorporation(s). Indeed, 0.3, 1.4 and 1.6 mg root dry matter cm$^{-3}$ (SD ±0.2, ±0.8 and ±0.3) were washed out from the bulk density samples of the top 15 cm for COM, MCF and MNF, respectively, without a significant difference between −P and +P treatments.

**Microbial P uptake in a lab experiment**

While the close relationship between soil C and microbial biomass C has been shown previously, the overriding C limitation of microbial P in a P deficient tropical soil is more surprising. However, microbial P uptake may become P limited when C substrates are abundant. We therefore investigated if the increase in microbial P after additions of a soluble C source was influenced by P fertilization. When small quantities of glucose (0.5 g C kg$^{-1}$) were added, $P_{\text{hex}}$ increased by 2 mg kg$^{-1}$ irrespective of the P status of the soil (Figure 1.4). Larger additions of 2.5 g C kg$^{-1}$ enhanced the initial immobilization of P by 5 and 9 mg kg$^{-1}$ for −P and +P treatments, respectively. Even higher microbial P uptake together with a greater difference between −P and +P treatments (23 and 41 mg P kg$^{-1}$) was observed when N was supplied together with C. Sole additions of N, however, did not affect $P_{\text{hex}}$ (data not shown). At high levels of C availability, microbial P uptake therefore appears to be limited primarily by N availability, while P fertilization affects only the maximal extent of P immobilization.
All C additions decreased $P_{\text{resin}}$ (Figure 1.4), illustrating the potential of microbial P immobilization to decrease P availability to plants. However, the $P_{\text{resin}}$ depletion amounted less than the increase in microbial P, suggesting that the microbial biomass took up P from other pools as well or that $P_{\text{resin}}$ was rapidly replenished. Upon release, P from microorganisms may result in increased levels of $P_\circ$ as observed by Chauhan et al. (1979) or eventually become available to plants.

**Conclusions**

Our results suggest that the regular inclusion of a one-season legume fallow into the continuous maize cultivation practiced by small-scale farmers in western Kenya has potential to increase maize production and simultaneously improve soil fertility by preventing losses of soil organic matter and increasing internal nutrient cycling. The system cannot, however, be successful if the pest problems observed with several improved fallow species are not overcome. The use of mixed species fallows and the preference of indigenous over introduced fallow species have been suggested as means to limit pest problems (Desaeger, 2001; Niang et al., 2002) but require further testing before recommendations can be made. For soils low in available inorganic but not in total P stocks, as was observed in this study, increased P cycling under fallows resulting
from a shift towards organic and microbial P may be a feasible alternative to large additions of inorganic P fertilizer. Nevertheless, additions of inorganic N and P in the order of 70 and 20 kg ha\(^{-1}\) yr\(^{-1}\), respectively, are required to level out nutrient balances, and inputs of other nutrients such as K will also be necessary. A better understanding of the availability of organically bound nutrients and the risk for nutrient immobilization to increase together with biological activity is still required and should be addressed through incubation and pot experiments as well as better monitoring of seasonal dynamics and the residual effects of crop rotations in the field.

**Acknowledgements**

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Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization
“Sugar makes the world go around”

_after R. Kelly (“Cabaret”)_
Abstract

A better understanding of soil microbial processes is required to improve the synchrony between nutrient release from plant residues and crop nutrient demand. Phospholipid fatty acid (PLFA) analysis was used to investigate the effect of two crop rotations (continuous maize and maize-crotalaria rotation) and P fertilization (0 and 50 kg P ha$^{-1}$ yr$^{-1}$, applied as triple superphosphate) on microbial community composition in a highly weathered soil from western Kenya. Microbial substrate use in soils from the field experiment was compared in incubation experiments. Higher levels of soil organic matter and microbial biomass in the maize-crotalaria rotation were connected with higher total amounts of phospholipid fatty acids and an increase in the relative abundances of indicators for fungi and gram-negative bacteria. P fertilization changed the community profile only within the continuous maize treatment. The decomposition of glucose, cellulose and three plant residues (all added at 2.5 g C kg$^{-1}$ soil) proceeded faster in soil from the maize-crotalaria rotation, but differences were mostly transient. Microbial P and N uptake within one week increased in relation to the water-soluble C content of added plant residues. More P and N were taken up by the microbial biomass in soil from the maize-crotalaria rotation than from continuous maize. In both soils, P fertilization increased microbial P uptake under conditions of high C availability after glucose additions. Re-mineralization of nutrients during the decline of the microbial biomass was greater in soil from the maize-crotalaria rotation but occurred only for the plant residue with the highest quality within the half year incubation period. Possible consequences for nutrient cycling in the field are discussed.
Introduction

Levels of soil organic matter, microbial biomass and soil respiration are increased by the introduction of a one-season fallow with a legume such as *Crotalaria grahamiana* Wight & Arn. (subsequently referred to as crotalaria fallow) into the traditional system of continuous maize cultivation practiced in western Kenya (Smestad et al., 2002 and chapter 1 of this thesis). Work on temperate soils suggests that the microbial utilization efficiency of freshly added plant residues can be affected by the initial size and activity of the soil microbial biomass (Fliessbach et al., 2000). Under tropical conditions, the microbial biomass may be able to respond very rapidly to new substrate inputs. In Nigeria, the leaf decomposition proceeded more slowly in a degraded than in a non-degraded Alfisol, but differences had disappeared after 150 days, although the degraded soil initially contained less than half as much microbial biomass (Tian, 1998).

Nevertheless, changes not only in the size but also in the structure of the microbial community could influence patterns of immobilization and mineralization after the addition of plant residues. Under conditions of low nutrient availability, the temporal matching of nutrient release from added organic materials with crop nutrient uptake is especially desirable, a concept known as synchrony (Myers et al., 1997). In the highly weathered soils of western Kenya, P is usually the primary limiting nutrient. It has been hypothesized that microbial immobilization of P after addition of organic material can prevent P from being sorbed. However, plant P availability will only be improved if the release of immobilized P is synchronized with plant P uptake, and it is therefore important to understand which factors affect microbial P dynamics. Results from a field experiment in western Kenya as well as glucose additions in laboratory incubations suggested that microbial P uptake was limited by C and N rather than P availability (chapter 1).

The main hypothesis for this study was that the rate of substrate degradation and changes in microbial P depend on the proportion of soluble C in added substrates as well as the initial properties of the microbial biomass and P availability. We therefore compared a) the composition of the microbial biomass as determined by analysis of phospholipid fatty acids (PLFA) in samples taken in a field experiment with two crop rotations (continuous maize and maize-crotalaria fallow rotation) at two levels of P fertilization (0 and 50 kg P ha\(^{-1}\) yr\(^{-1}\), applied as triple superphosphate) on a highly weathered soil, and b) the decomposition of model C substrates and plant residues.
added to these soils as well as resulting microbial P dynamics in incubation experiments. In the experiment with plant residues, microbial C and N were also measured, as it is not known whether they change in parallel with microbial P. Ratios of C and P in the microbial biomass have been reported to be highly variable in incubation experiments (Chauhan et al., 1981) and during seasonal changes in the field (He et al., 1997), but evidence from tropical soils is scarce.

**Methods**

*Soil characteristics*

Soil samples were taken in a field experiment in western Kenya (0°09’ N, 34°33’ E) on a kaolinitic, isohyperthermic Kandiudalfic Eutrudox (USDA classification) or a Ferralsol (FAO) with 39% clay and 37% sand in the top 15 cm. In this experiment, different crop rotations (continuous maize and various maize-fallow rotations) and P fertilization rates have been compared since 1997. The treatments selected for the present study are shown in Table 2.1. Continuous maize (COM) represents the traditional system with two maize crops per year, whereas the rotation of maize with a crotalaria fallow (MCF) is tested as an option for soil fertility improvement. In both systems, the cumulative maize grain yield of 5.5 years was doubled by P fertilization. Maize yield was also slightly higher in MCF, although there was only one maize crop per year compared to two in COM. Further details of the field experiment are given in chapter 1.

In January 2000, 2001 and 2002, respectively, a composite soil sample consisting of 15 random cores was collected from each plot (0-15 cm). At this time, maize had just been harvested while the fallows of the third, fourth and fifth fallow phase, respectively, were still standing. Field-moist samples were sieved at 4 mm to remove coarse plant debris, and stored at 4°C until the set up of the incubation experiments. The selected treatments did not significantly affect soil pH (5.0), CEC (5.8 cmolc kg⁻¹) and base saturation (71%). P fertilization increased resin-extractable P (P_{resin}) as well as total P (P_{tot}), but did not affect organic C and N (C_{tot} and N_{tot}) nor microbial C, N and P (C_{chi}, N_{chi}, and P_{chi}), which were higher under MCF than under COM (Table 2.1). These results were highly reproducible at the different times of sampling as shown for microbial C and P (Table 2.1).
Table 2.1: Field treatments selected for this study and effect on maize yield, chemical and biological soil properties

<table>
<thead>
<tr>
<th>Field treatment</th>
<th>P input $^b$</th>
<th>crop</th>
<th>total maize grain yield</th>
<th>$P_{\text{resin}}$</th>
<th>$P_{\text{tot}}$</th>
<th>$C_{\text{tot}}$</th>
<th>$N_{\text{tot}}$</th>
<th>$N_{\text{min}}$ $^c$</th>
<th>$N_{\text{chl}}$</th>
<th>$C_{\text{chl}}$</th>
<th>$P_{\text{hex}}$</th>
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<tr>
<td></td>
<td>kg ha$^{-1}$ yr$^{-1}$</td>
<td></td>
<td>1997-2002</td>
<td>‘00</td>
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<td>‘00</td>
<td>‘01</td>
<td>‘02</td>
<td>‘00</td>
</tr>
<tr>
<td>COM–P</td>
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<td>maize</td>
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<td>23.7</td>
<td>1.6</td>
<td>58</td>
<td>98</td>
<td>96</td>
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<td>maize</td>
<td>17.7</td>
<td>6.9</td>
<td>838</td>
<td>23.4</td>
<td>1.6</td>
<td>58</td>
<td>97</td>
<td>96</td>
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<tr>
<td>MCF–P</td>
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<td>fallow</td>
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<td>721</td>
<td>26.2</td>
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<td>fallow</td>
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<td>829</td>
<td>27.4</td>
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<td>155</td>
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</table>

Source of variation

- crop rotation
- P fertilization
- crop rotation x P fertilization

* $P = 0.01-0.05$, ** $P = 0.01-0.001$, *** $P < 0.001$, ns = not significant

* analysis performed on each of the four field replications

$^b$ applied as triple superphosphate to the maize crop in season 1

$^c$ mineral N (sum of ammonium and nitrate) extracted with 2 M KCl

$^d$ $P = 0.051$
**Incubation experiments**

Two incubation experiments were conducted (Table 2.2). Before each experiment, equal amounts of soil from each of the four field replications of the same treatment were thoroughly mixed to give a composite sample and preconditioned at 25% water content (60% water holding capacity) at 25°C for 12 days in the dark. Substrate-amended soils and the non-amended controls in both experiments were incubated at 25°C in closed bottles (250 ml) containing a NaOH trap for soil respiration measurement and in perforated polyethylene bags for microbial extractions. Water losses were adjusted gravimetrically every week.

<table>
<thead>
<tr>
<th>exp. year</th>
<th>soils</th>
<th>COM</th>
<th>MCF</th>
<th>amendments</th>
<th>analyses</th>
<th>duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 00</td>
<td>X X X X</td>
<td>glucose + N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>X X</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>2 01</td>
<td>X X</td>
<td>cellulose + N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CO&lt;sub&gt;2&lt;/sub&gt;, C&lt;sub&gt;chl&lt;/sub&gt;, N&lt;sub&gt;chl&lt;/sub&gt;, N&lt;sub&gt;K:SO&lt;sub&gt;4&lt;/sub&gt;&lt;/sub&gt;</td>
<td>X X X X</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2: Overview of the incubation experiments

<sup>a</sup> added at 2.5 mg C g<sup>-1</sup> soil
<sup>b</sup> added as NH<sub>4</sub>N O<sub>3</sub> at 0.25 mg N g<sup>-1</sup> soil

**Experiment 1**

Soil samples from the field treatments COM–P, COM+P, MCF–P and MCF+P were thoroughly mixed with two different carbon substrates: a) glucose and b) crystalline cellulose (Avicel™), both added at 2.5 mg C g<sup>-1</sup> soil together with NH<sub>4</sub>N O<sub>3</sub> at 0.25 mg N g<sup>-1</sup> soil. In the case of glucose, the release of CO<sub>2</sub> was measured at 8 dates during 28 days and changes in microbial P on day 2, 4, 7, 14 and 28 after substrate amendment. In the case of cellulose, CO<sub>2</sub>-release was determined at 19 dates during 112 days of incubation, while microbial P determinations (day 2, 7, 14, 22, 28, 36 and 46) were discontinued after day 46 due to very small and often insignificant treatment effects.
Experiment 2

Three plant residues were used (crotalaria, maize stover, maize roots). Above-ground crotalaria residues (excluding wood) which in the following are termed ‘crotalaria’, and maize stover were obtained from a field experiment in western Kenya (Gathumbi et al., 2002). Maize roots were washed out from a pot experiment with maize (Zea mays L. cv. Corso) grown for 60 days in a mixture of soil and sand in a volume-based ratio of 1:4 (Jansa, 2002). Due to the cultivar and growing conditions, these maize roots are probably not representative of maize roots growing in the field in Kenya, but were included as an additional residue of different quality than crotalaria and maize stover. All residues were air-dried, ground to pass a 0.5 mm sieve and added to the soils MCF-P and COM-P at 2.5 mg C g\(^{-1}\) soil. The released CO\(_2\) was measured at 16 dates during 183 days. Extractions for microbial P, C and N were done on day 7, 21, 56 and 183. Microbial P was additionally determined on day 14 and 112. Two of the residues (crotalaria, maize stover) were also used after leaching of the water-soluble fraction. They were added to both soils at 2.5 mg C g\(^{-1}\) soil and the release of CO\(_2\) was determined at the same dates as for the other residues during the 183-day incubation.

Phospholipid fatty acid analysis

Details of PLFA analysis are given in Bossio and Scow (1998). Briefly, lipids were extracted for 2 h with a chloroform:methanol:phosphate buffer (Bligh and Dyer, 1959) from unsieved soil samples (8 g dry weight) taken in 2002 and frozen as soon as possible after sampling. Phospholipids were then separated from neutral lipids and glycolipids on solid phase extraction columns, 0.50 g Si (Supelco, Inc., Bellefonte, Penn), and subjected to mild alkaline methanolysis (Dowling et al., 1986) to recover fatty-acid methyl esters. Samples were analyzed using a gas chromatograph (Hewlett Packard 6890), and peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE). Peak identification was verified by mass spectrometry.

Fatty acids were designated in the form “x:y”, where x is the total number of carbon atoms and y the number of double bonds (unsaturations), followed by ω before the position of the double bond from the methyl end of the molecule. The suffixes c and t indicate cis and trans geometry, and the prefixes a and i refer to anteiso- and iso-
branching. 10me indicates a methyl group on the tenth carbon atom from the carboxyl end of the molecule. Positions of cyclopropyl groups (cy) groups are noted.

The percentage of the total amount of PLFAs extracted (i.e. the relative abundance) was compared for selected signature fatty acids and structural groups of fatty acids. Linoleic acid (18:2ω6c) is a biomarker for fungi (Federle, 1986). A methyl branching on the tenth C atom (10me16:0, 10me17:0, 10me18:0) indicates actinomycetes (Kroppenstedt, 1985). Frostegård and Bååth (1996) chose a set of fatty acids to represent bacterial PLFAs, out of which i15:0, a15:0, 15:0, i16:0, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7, and cy19:0 were present in our samples. Mono-unsaturated fatty acids are considered indicative of gram-negative bacteria, although they also occur in gram-positive bacteria (Zelles, 1999). In our study, the following mono-unsaturated fatty acids were detected: 16:1ω7t, 16:1ω11c, 16:1ω7c, 16:1ω5c, 17:1ω9c, 17:1ω8c, 17:1ω7c, 17:1ω5c, 18:1ω9c, and 18:1ω7c. Although α- or i-branched saturated fatty acids also occur in some gram-negative organisms, they are mainly representative of gram-positive bacteria (Haack et al., 1994). We show the sum of i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0, and i19:0. Finally, fatty acids with cyclopropyl groups (17:0cy and 19:0cy) increase during the stationary growth phase, indicating starvation conditions (Law et al., 1963), but are also most common in gram-negative strains (Zelles, 1999).

**Soil respiration and microbial extractions**

Soil respiration measurements and microbial extractions were done in triplicate. The CO₂ released from incubated soils (20 g) was trapped in 20 ml of 0.05 or 0.1 M NaOH and determined by titration with 0.1 or 0.2 M HCl (Alef, 1995). P held in the microbial biomass was determined by simultaneous liquid fumigation and extraction with anion-exchange resin membranes (BDH #55164) in bicarbonate form for 16 h as described by Kouno et al. (1995), but using hexanol as the fumigant instead of chloroform which was found to dissolve these anion-exchange membranes. Fumigation with hexanol has been shown to be as effective as chloroform fumigation to release microbial P (McLaughlin et al., 1986). Subsamples with addition of an inorganic P spike equal to 5 mg P kg⁻¹ were included in order to account for sorption of P released during the extraction period. The concentration of P in all extracts was determined colorimetrically (Murphy and Riley, 1962). C and N held in the microbial biomass were determined by 24 h
Table 2.3: Properties of plant residues used in the incubation experiment 2

<table>
<thead>
<tr>
<th></th>
<th>crotalaria</th>
<th>maize stover</th>
<th>maize roots</th>
<th>crotalaria leached</th>
<th>maize stover leached</th>
</tr>
</thead>
<tbody>
<tr>
<td>total plant material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{tot}}$</td>
<td>g kg$^{-1}$</td>
<td>427 a, AB</td>
<td>412 b, B</td>
<td>341 c, C</td>
<td>337 C</td>
</tr>
<tr>
<td>ash content</td>
<td>%</td>
<td>8.1 c, C</td>
<td>9.4 b, B</td>
<td>28.5 a, A</td>
<td>6.7 D</td>
</tr>
<tr>
<td>(lignin+polyphenol):$N_{\text{tot}}$</td>
<td>%</td>
<td>10 b, D</td>
<td>59 a, B</td>
<td>55 a, B</td>
<td>15 C</td>
</tr>
<tr>
<td>$C_{\text{H}<em>2O}:N</em>{\text{tot}}$</td>
<td></td>
<td>2.9 b</td>
<td>3.7 b</td>
<td>9.8 a</td>
<td>nd</td>
</tr>
<tr>
<td>$C_{\text{tot}}:P_{\text{tot}}$</td>
<td>% of $P_{\text{tot}}$</td>
<td>48 b</td>
<td>42 b</td>
<td>77 a</td>
<td>nd</td>
</tr>
<tr>
<td>soluble nutrients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{H}_2O}$</td>
<td>% of $C_{\text{tot}}$</td>
<td>27 a</td>
<td>7 c</td>
<td>17 b</td>
<td>nd</td>
</tr>
<tr>
<td>$N_{\text{H}_2O}$</td>
<td>% of $N_{\text{tot}}$</td>
<td>38 a</td>
<td>28 b</td>
<td>35 ab</td>
<td>nd</td>
</tr>
<tr>
<td>$P_{\text{resin}}$</td>
<td>% of $P_{\text{tot}}$</td>
<td>60 a</td>
<td>53 b</td>
<td>39 c</td>
<td>nd</td>
</tr>
<tr>
<td>$P_{\text{resin}}$ fumigated</td>
<td>% of $P_{\text{tot}}$</td>
<td>64 a</td>
<td>61 a</td>
<td>40 b</td>
<td>nd</td>
</tr>
<tr>
<td>added amounts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N_{\text{tot}}$</td>
<td>mg kg$^{-1}$ soil</td>
<td>240 a, A</td>
<td>43 b, CD</td>
<td>45 b, C</td>
<td>168 B</td>
</tr>
<tr>
<td>$P_{\text{tot}}$</td>
<td></td>
<td>14.2 a, A</td>
<td>3.9 c, D</td>
<td>5.7 b, C</td>
<td>6.8 B</td>
</tr>
</tbody>
</table>

Within rows, means followed by the same letter are not significantly different ($P = 0.05$) by Tukey’s multiple range test (small and capital letters indicate differences between non-leached and all residues, respectively); nd: not determined.
fumigation followed by extraction with 0.5 M K$_2$SO$_4$ as described by Vance et al. (1987), with measurement of total C and N in the extracts using a Dimatoc 100 apparatus (Dimatec, Essen, Germany). Microbial P, C and N are reported as amounts rendered extractable by hexanol (P$_{hex}$) and chloroform (C$_{chl}$ and N$_{chl}$) fumigation, respectively, without the use of a conversion factor. Amounts of P and N extracted from unfumigated subsamples are reported as resin-extractable P (P$_{resin}$) and K$_2$SO$_4$-extractable N (N$_{K_2SO_4}$), respectively. All treatment effects (CO$_2$, P$_{hex}$, C$_{chl}$, N$_{chl}$, P$_{resin}$, N$_{K_2SO_4}$) are presented as the difference between amended soils and the non-amended controls.

Plant residue properties

Plant residue characteristics were analyzed with three to five analytical replicates. Total C and N (C$_{tot}$, N$_{tot}$) were determined on ball-milled subsamples using a CN analyzer (Carlo Erba Instruments, NA 1500, Rodano-Milano, Italy). The ash content was determined after ignition at 550°C. Total P (P$_{tot}$) was determined colorimetrically after dissolving the ash in 2 ml 20% HCl, making to 50 ml volume with H$_2$O$_{dest}$ and filtering (Whatman no. 40). For the determination of water soluble C (C$_{H_2O}$) and N (N$_{H_2O}$), 0.2 g residue was shaken with 30 ml H$_2$O$_{dest}$ for 16 h, vacuum-filtered through DURAN® glass filters (size 2), and C and N in the extracts measured with the Dimatoc apparatus mentioned above. P$_{resin}$ was determined by extracting 0.05 g residue with 6 anion-exchange membranes in 30 ml H$_2$O similar to the method for P$_{hex}$. This extraction was done with or without 1 ml hexanol in order to test if P released from plant material in the presence of hexanol would affect the determination of microbial P. Lignin and polyphenol contents of the residues were determined as described by Anderson and Ingram (1993).

The properties of plant residues used in experiment 2 are shown in Table 2.3. The high ash content of maize roots indicates a contamination by the growth substrate, but this was overcome by adding all residues at a constant amount of C. Low C:N- and C:P-ratios as well as high proportions of soluble carbon classify crotalaria as a high quality residue. Maize stover and maize roots have much wider C:N-ratios that are similar to each other, while C:P-ratios become wider and the proportion of soluble C smaller in the order crotalaria, maize roots and maize stover. Leaching of crotalaria and maize stover widens the C:N- and C:P-ratios. Except for maize stover, the ratio of (lignin+polyphenol):N is narrow. The proportion of P$_{resin}$ ranges between 39-60% of
P_{tot}, depending on the residue, and is significantly higher in the presence of hexanol, except in the case of maize roots. This increase in P_{resin} translates into 0.5, 0.3 and 0.1 mg P kg^{-1} soil for crotalaria, maize stover and maize roots, respectively, which could be rendered extractable by hexanol fumigation and misinterpreted as an increase in P_{hex}.

**Statistical analysis**

PLFA profiles (i.e. the patterns of relative abundances of individual fatty acids) were analyzed with CANOCO software (Microcomputer Power, Inc., Ithaca, NY). Out of the 51 detected PLFAs, a subset of 43 was chosen for statistical analysis, excluding those that occurred in less than half the samples or were unreliably quantified due to concentrations near the detection limits. Principal component analysis (PCA) and redundancy analysis (RDA) were applied. PCA is used to explore patterns in multivariate data sets by creating axes that encompass the greatest amount of variability possible, thus reducing the dimensions in the data. RDA is a direct ordination technique based on PCA, in which ordination axes are constrained to be linear combinations of environmental variables, thus allowing direct testing of the significance of treatments (ter Braak, 1987). RDA analysis was done with crop rotation and P fertilization as environmental variables. Significance testing of these factors in terms of explaining variation in the multivariate data set was accomplished using the Monte Carlo permutation test (ter Braak, 1990) available in the CANOCO software.

The remaining statistical analyses were carried out with SYSTAT (SPSS 2000). Relative abundances of signature fatty acids and structural groups were tested by three-way ANOVA with the factors field replication, crop rotation, P fertilization and the interaction between crop rotation and P fertilization, followed by Tukey’s multiple comparison test whenever significant differences ($P \leq 0.05$) were indicated. Respiration and microbial data at single dates and for a given substrate within experiment 1 were analyzed by two-way ANOVA with the factors crop rotation, P fertilization and their interaction. In experiment 2, soil effects at single dates and for a given residue were analyzed with a t-test. In both experiments, t-tests were also performed at single dates to check for significant differences between each treatment and its non-amended control. Nutrient ratios of plant residues and in the microbial biomass were calculated by pairing replicates by chance. Before the statistical analysis, nutrient ratios were log-transformed and fractions (percentage/100) arcsin-transformed.
Results

PLFA profiles and signature fatty acids

PLFA profiles from the two crop rotations COM and MCF were significantly separated ($P < 0.01$) along a first PCA axis that encompassed 39% of the total variability in the profiles (Figure 2.1). In addition, significant differences ($P < 0.05$) were observed between +P and -P plots within COM but not within MCF. The most abundant PLFAs were $i15:0$, 16:0 and $18:2\omega9c$, each amounting to 10-14% of the total amount of PLFAs extracted, while another 23 PLFAs had a relative abundance of <1% each.

![Figure 2.1: Principal component analysis of PLFA profiles](image)

Higher total amounts of PLFAs were extracted from MCF than from COM (Table 2.4), and there was a trend towards increased total amounts of PLFAs due to P fertilization ($P = 0.055$). The greater microbial biomass in MCF was accompanied by an increase in the relative abundance of the fungal indicator ($18:2\omega6c$). The sets of fatty acids chosen to represent actinomycetes and bacteria both separated the treatment COM–P from the three other treatments, although the interaction between crop rotation and P fertilization was not significant ($P = 0.077$ and 0.055, respectively). The relative abundances of mono-unsaturated fatty acids indicating gram-negative bacteria increased in the order COM–P, COM+P, MCF–P, and MCF+P, while the α- or β-branched fatty acids chosen...
as a biomarker for gram-positive bacteria decreased in the same order. Fatty acids with a cyclopropyl group which may indicate conditions of starvation or the abundance of some gram-negative organisms were not affected by the crop rotations but were relatively increased by P fertilization.

Table 2.4: Total amount of PLFAs and relative abundance of selected signature fatty acids and structural groups as affected by crop rotation and P fertilization

<table>
<thead>
<tr>
<th>field treatment</th>
<th>total amount of signature fatty acids and structural groups</th>
<th>relative abundance (mol%) of signature fatty acids and structural groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol g⁻¹ soil</td>
<td>fungi⁵</td>
</tr>
<tr>
<td>COM–P</td>
<td>47.6 c</td>
<td>0.70 b</td>
</tr>
<tr>
<td>COM+P</td>
<td>49.2 bc</td>
<td>1.03 b</td>
</tr>
<tr>
<td>MCF–P</td>
<td>63.5 ab</td>
<td>1.84 a</td>
</tr>
<tr>
<td>MCF+P</td>
<td>77.1 a</td>
<td>1.84 a</td>
</tr>
</tbody>
</table>

source of variation

- crop rotation (R)
- P fertilization (P)
- R x P

Within columns, means followed by the same or no letter are not significantly different (P = 0.05) by Tukey’s multiple range test

a 18:2ω6c (Federle 1986)
\[ \sum (10ω6; 10ω7; 10ω8) \] (Kroppenstedt 1985)

b selected bacterial fatty acids (Frostegård and Bååth 1996)

c monounsaturated fatty acids (Zelles 1999)

d a-/i-branched fatty acids (Haack et al. 1994)

e fatty acids with a cyclopropyl group (Law et al. 1963, Zelles 1999)

Experiment 1: glucose and cellulose added to MCF±P and COM±P

The initial release of CO₂ after glucose addition proceeded faster in P-fertilized soils, with maximum daily respiration rates of up to 500 mg C kg⁻¹ day⁻¹ measured on day 3 for +P and on day 4 for −P treatments (Figure 2.2a). During the first three days, the cumulative CO₂-release was also higher in MCF than in COM (at both levels of P fertilization). From day 7 onwards, the cumulative CO₂-release was similar in all soils, amounting to 65-66% of the added C at the end of the incubation. The degradation of
cellulose proceeded four to five times slower than the degradation of glucose, depending on the soil (Figure 2.2b). From day 2 onwards and in all soils, cellulose additions significantly increased CO2-release compared to the non-amended controls, in which respiration rates remained below 5 mg C kg⁻¹ day⁻¹ after the first week. In the cellulose-amended soils, there was a lag time of 9 to 30 days until respiration rates surpassed 10 mg C kg⁻¹ day⁻¹, depending on the soil. Maximum CO2-release rates were measured on day 16, 23, 38 and 42 and amounted to 55, 48, 29 and 27 mg C kg⁻¹ day⁻¹ for MCF+P, MCF−P, COM+P and COM−P, respectively. After 112 days, 59-70% of the C added with cellulose had been mineralized. The cumulative CO2-release from the non-amended controls amounted to 175, 210, 325, and 362 mg C kg⁻¹ for COM−P, COM+P, MCF−P, and MCF+P, respectively.

Figure 2.2: Cumulative CO2-release from soils amended with glucose (a) or cellulose (b); bars show HSD₀.₀₅ for dates at which significant differences between treatments occurred; final data points followed by the same letter (or ns) are not significantly different (P = 0.05) by Tukey’s test.

Two days after the addition of glucose, the microbial biomass had taken up 17-41 mg P kg⁻¹, depending on the soil (Figure 2.3a). Except for the last sampling date, the change in Phex compared to the non-amended controls was significantly greater in MCF than in COM, whereas P fertilization increased only the initial P uptake. Most of the P taken up by the microbial biomass had been released again after 28 days, when Phex was 5-8 mg P
kg⁻¹ higher in amended than in control soils. Cellulose additions resulted in few significant changes in P_{hex} with a maximum of 1.8 mg P kg⁻¹ in COM+P (Figure 2.3b). Neither P fertilization nor crop rotations had a consistent effect on P_{hex}, except that between day 22 and 46, the greatest change in P_{hex} was measured in COM+P.

![Figure 2.3: Change in P_{hex} in soils amended with glucose (a) or cellulose (b); bars show HSD₀.₀₅ for dates at which significant differences between treatments occurred; dotted symbols indicate non-significant changes compared to the non-amended control.](image_url)

Figure 2.4: Change in P_{resin} in soils amended with glucose (a) or cellulose (b); bars show HSD₀.₀₅ for dates at which significant differences between treatments occurred; dotted symbols indicate non-significant changes compared to the non-amended control.
Glucose additions significantly decreased \( P_{\text{resin}} \) in all soils, with greater absolute reductions of up to 6 mg P kg\(^{-1}\) observed in +P treatments (Figure 2.4a). Relative to the initial values, \( P_{\text{resin}} \) had decreased by 80-90% in all soils after two days. Subsequently, \( P_{\text{resin}} \) increased again, but at the last sampling, it was still 40-60% lower than in the control soils. Contrary to changes in \( P_{\text{hex}} \), there was no consistent effect of the crop rotation on changes in \( P_{\text{resin}} \). After addition of cellulose, \( P_{\text{resin}} \) initially increased by 0.4-1.1 mg P kg\(^{-1}\) but subsequently decreased again to values that were similar to or 2-4 mg P kg\(^{-1}\) lower than the non-amended controls for −P and +P treatments, respectively (Figure 2.4b). The greatest decrease in \( P_{\text{resin}} \) was measured in COM+P, the soil for which the maximum increase in \( P_{\text{hex}} \) was observed.

**Experiment 2: plant residues added to MCF−P and COM−P**

Because in experiment 1, the effect of crop rotations on microbial dynamics was generally larger than the effect of P fertilization, only soils MCF−P and COM−P were used in experiment 2. For the three non-leached residues, the amount of CO\(_2\) released during the first two days after addition ranged in the order crotalaria > maize roots > maize stover (Figure 2.5a-c). Significant differences between soils MCF−P and COM−P occurred after addition of all residues, but in the case of maize roots, they did not amount to more than 54 mg C kg\(^{-1}\) and had vanished completely after 21 days. In the case of maize stover, differences were larger (max. 160 mg C kg\(^{-1}\)), but still transient. In contrast, the crop rotation effect on degradation of crotalaria persisted until the end of the experiment, when an additional 275 mg C kg\(^{-1}\) had been released from MCF−P compared to COM−P. In the case of crotalaria, leaching of the soluble fraction decreased the difference between soils to non-significant values from day 67 onwards (Figure 2.5d), whereas it increased the difference in the case of maize stover (Figure 2.5e). The greatest difference between soils in CO\(_2\)-release from maize stover was observed after 123 days (381 mg C kg\(^{-1}\)), but diminished towards the end of the experiment (354 mg C kg\(^{-1}\)). The apparent release of added C at the end of the incubation ranged between 37% (leached maize stover added to COM−P) and 64% (crotalaria added to MCF−P).
Figure 2.5: Cumulative CO₂-release from soils amended with plant residues; * indicate dates with significant differences ($P = 0.05$) between soils according to a t-test.
Two days after addition of residues, changes in $P_{\text{hex}}$ amounted to a maximum of 8 mg P kg$^{-1}$, ranging in the order crotalaria > maize roots > maize stover (Figure 2.6a-c). A significant decrease towards the last sampling point was observed for crotalaria, while $P_{\text{hex}}$ increased or remained constant in the case of maize stover and roots, respectively. The potential release of 0.5, 0.3 and 0.1 mg P kg$^{-1}$ upon fumigation from crotalaria, maize roots and maize stover, respectively, represents a small source of error compared to the observed changes in $P_{\text{hex}}$ and would have been similar for both soils. Generally, larger changes in $P_{\text{hex}}$ occurred in MCF–P than in COM–P, with an additional P uptake of up to 3.5 mg P kg$^{-1}$. In contrast, changes in $P_{\text{resin}}$ were in most cases similar in both soils. The addition of crotalaria decreased $P_{\text{resin}}$ by 0.2-0.4 mg P kg$^{-1}$ during the first 6 weeks but led to a subsequent increase in $P_{\text{resin}}$ by up to 1 mg P kg$^{-1}$ above the non-amended controls (Figure 2.6a). The addition of maize stover did not significantly change $P_{\text{resin}}$ (Figure 2.6b), while the addition of maize roots caused a continuing decrease in $P_{\text{resin}}$ by 0.3-0.7 mg P kg$^{-1}$ (Figure 2.6c).

After one week, the addition of plant residues had increased $N_{\text{chl}}$ by 4-14 mg N kg$^{-1}$, with the increase ranging in the order crotalaria > maize roots > maize stover (Figure 2.7). At most dates, maize stover and maize roots confirmed the trend observed for $P_{\text{hex}}$ of greater changes in MCF–P (Figure 2.7b, c), but in the case of crotalaria, $N_{\text{chl}}$ was higher in COM–P than in MCF–P at day 21 and not significantly different from the controls thereafter (Figure 2.7a). The addition of maize stover and maize roots caused a prolonged reduction in $N_{\text{K2SO4}}$ which was greater in MCF–P than in COM–P (Figure 2.7b, c), whereas the addition of crotalaria immediately increased $N_{\text{K2SO4}}$, with higher final values in MCF–P than in COM–P (Figure 2.7a).

Similar to $P_{\text{hex}}$ and $N_{\text{chl}}$, $C_{\text{chl}}$ also increased after addition of all plant residues, with the change ranging in the order crotalaria > maize roots > maize stover and MCF–P > COM–P (data not shown). Relative to initial values, however, $P_{\text{hex}}$ changed to a greater extent than $C_{\text{chl}}$. Therefore, the $C_{\text{chl}}$:$P_{\text{hex}}$-ratio of the extractable microbial biomass was generally narrowed by residue addition, although not always significantly (Figure 2.8).

Time trends after residue addition were similar in both soils, but the $C_{\text{chl}}$:$P_{\text{hex}}$-ratio remained narrower in MCF–P than in COM–P, as was observed in the non-amended controls. In contrast, the $C_{\text{chl}}$:$N_{\text{chl}}$-ratio was generally not significantly different from the controls, indicating similar changes in $C_{\text{chl}}$ and $N_{\text{chl}}$ (Figure 2.8).
Figure 2.6: Changes in $P_{\text{hex}}$ and $P_{\text{resin}}$ in soils amended with plant residues; * between and below symbols for $P_{\text{hex}}$ and $P_{\text{resin}}$, respectively, indicate dates with significant differences ($P = 0.05$) between soils according to a t-test; dotted symbols indicate non-significant changes compared to the non-amended control.
Figure 2.7: Changes in N$_{chl}$ and N$_{K_2SO_4}$ in soils amended with plant residues; * above and below symbols for N$_{chl}$ and N$_{K_2SO_4}$, respectively, indicate dates with significant differences ($P = 0.05$) between soils according to a t-test; dotted symbols indicate non-significant changes compared to the non-amended control.
Figure 2.8: Microbial nutrient ratios ($C_{chl}:P_{hex}$ and $C_{chl}:N_{chl}$) in soils amended with plant residues; * between and below symbols for $C_{chl}:P_{hex}$ and $C_{chl}:N_{chl}$, respectively, indicate dates with significant differences ($P = 0.05$) between soils according to a t-test; values at day 0 are averages for non-amended controls; dotted symbols indicate non-significant changes compared to the non-amended control.
Discussion

Size and composition of the microbial community
Higher total amounts of PLFAs extracted from MCF than from COM confirmed observations using fumigation-extraction methods that the size of the microbial biomass differed between the two crop rotations. The total amount of PLFAs was significantly correlated to microbial P ($r^2 = 0.77$), C ($r^2 = 0.70$) and N ($r^2 = 0.54$), respectively ($n = 16$). PLFA profiles additionally revealed changes in the composition of the microbial community which were primarily related to the effect of crop rotations (Figure 2.1). Increased C availability in MCF was accompanied by a doubling in the relative abundance of the fungal indicator and an increase in gram-negative bacteria as indicated by mono-unsaturated fatty acids (Table 2.4). This is in accordance with observations on the effect of rice straw incorporation (Bossio and Scow, 1998). High C availability resulting from the application of a synthetic root exudate (Griffiths et al., 1999) also led to a great increase in the abundance of the fungal indicator, while the abundances of actinomycetes, gram-positive bacteria, and the bacterial fatty acids selected by Frostegård and Bååth (1996) were decreased at high C application rates. The same tendency was observed in our study although COM+P was not significantly different from both MCF treatments in spite of different soil organic C contents (Table 2.1). In both crop rotations, the abundance of cyclopropyl fatty acids suggested as an indicator for starving conditions or gram-negative bacteria was similar between crop rotations but increased with P fertilization. However, the whole profile of the community differed between–P and +P plots only within COM (Figure 2.1).

While PLFA can detect differences in the size of the microbial biomass and differentiate between community fatty acid profiles, it is not possible to determine which organisms account for the differences, as unique fatty acids are lacking for most taxa (Haack et al., 1994). PLFA analysis can not distinguish between taxonomic and physiological changes in the community, nor be used to predict whether these changes affect microbial functions in soil. In the following sections, we discuss the results from our incubation experiments in relation to observed differences in the size and composition of the microbial community and to the quality of added substrates.
**CO$_2$-release**

Whenever the cumulative CO$_2$-release from added substrates differed between crop rotations, more CO$_2$ was released from MCF–P than from COM–P (Figures 2.2 and 2.5). A maximum difference of 770 mg C kg$^{-1}$ was observed in the case of cellulose after 40 days. It is clear that for all additions, positive priming effects on the mineralization of native soil organic matter or on the turnover of the native microbial biomass may have contributed to apparent differences between MCF and COM, as the extent of priming effects increases with soil organic matter contents (Kuzyakov et al., 2000). However, priming effects are usually strongest during the first 4-10 days after addition of substrates and have not been reported after addition of slowly decomposable substances (Hamer and Marschner, 2002). In our discussion, the comparison of CO$_2$-release between soils is used as an indicator of microbial substrate degradation in different soils, irrespective of potential priming effects.

In contrast to glucose and cellulose, plant residues are complex mixtures of C substrates of differing quality. This is also illustrated by the variable effect of leaching, which increased or decreased differences in CO$_2$-release between the two soils for maize stover and crotalaria, respectively (Figure 2.5). For wheat straw, Reinertsen et al. (1984) showed that water-soluble and intermediately-available C are metabolized together before structural components are attacked. In our study, the cumulative CO$_2$-release during the first week was closely related to (and not significantly different from) the amount of CH$_2$O added with crotalaria, maize stover and maize roots (data not shown), suggesting that during the first week, little mineralization of non-soluble, intermediately available C as proposed by Reinertsen et al. (1984) occurred.

As glucose is soluble and cellulose resembles structural components, some of the findings from experiment 1 can be used to interpret the degradation of plant residues. Similar to glucose, soluble substrates were degraded more rapidly in MCF than in COM during the first few days after addition (Figure 2.5). As glucose can be mineralized by most aerobic microorganisms (Anderson and Domsch, 1978), this effect appears to be related to the size rather than the composition of the microbial community. Likewise, Witter and Kanal (1998) observed the mineralization of glucose to increase with the initial amount of microbial biomass. In contrast, mineralization rates during the first two weeks after straw addition were not related to the size of the microbial biomass in the
study by Fliessbach et al. (2000), and the authors attributed this to the use of easily available compounds by rapidly-growing microorganisms. The initially more rapid degradation of glucose in P fertilized soils could then be attributed to improved growth conditions at higher P availability, as the initial size of the microbial biomass and basal respiration in the soils used in this experiment (sampled in 2000) did not differ between -P and +P treatments (Tables 2.1 and 1.7).

In the study by Fliessbach et al. (2000), 58% of a 14C-labeled residue incorporated into a bio-dynamically cropped soil had been mineralized after 177 days, compared to 50% in two conventionally cropped soils and an unfertilized control, which were all characterized by lower levels of microbial biomass than the bio-dynamically cropped soil. Different decomposition rates between day 16 and 86 were attributed to a faster decomposition of recalcitrant compounds in the bio-dynamically cropped soil. In our study, crystalline cellulose was used as a model structural C compound. Its degradation requires complex cellulase systems, the synthesis of which is repressed in the presence of low molecular-weight carbon sources and induced in the presence of cellulose (Béguin and Aubert, 1994). In cultures of the cellulolytic fungus *Volvariella volvacea* grown with Avicel™, a lag phase of 48 h was observed before the activities of endo-1,4-β-glucanase, cellulbiohydrolase, and β-glucosidase increased sharply (Cai et al., 1999). This agrees with our observation of a lag phase after addition of cellulose, which was prolonged in soils with a smaller initial biomass and less available P.

Changes in the composition of the microbial biomass, particularly the difference in the relative abundance of fungi, may also be responsible for the different rates of cellulose degradation. Although the ability to degrade cellulose is found among fungi and bacteria, cellulolytic fungi appear to produce more effective cellulase systems (Teeri, 1997). In addition, 86% of the carboxymethylcellulase activity in a forest soil was shown to be of fungal origin (Rhee et al., 1987). The amount of fungal indicator (18:2ω6c) extracted was 1.4, 1.2, 0.5, and 0.3 (SD ± 0.4, 0.2, 0.1 and 0.1) nmol g⁻¹ soil for MCF+P, MCF-P, COM+P and COM–P, respectively, and the time course of cellulose degradation (Figure 2.2b) as well as the relative abundance of the fungal indicator (Table 2.4) followed a similar treatment order. It is therefore not possible to decide whether differences in cellulose degradation were caused by differences in the size and/or composition of the microbial community.
Similar to cellulose, significant but transient differences between soils were observed during the decomposition of leached and non-leached maize stover, which had the lowest proportion of soluble C (Table 2.3). In the case of crotalaria, MCF apparently responded more rapidly to soluble as well as structural components of the residue. More complete degradation in MCF could also point to an adaptation of the microbial community to some components of crotalaria (due to previous inputs in the field) which were removed by leaching (Figure 2.5d).

**Microbial nutrient uptake and release**

From their experiments with wheat straw containing different amounts of soluble C, Reinertsen et al. (1984) concluded that the extent of N immobilization depends on the amount of soluble and intermediately-available C in the residue. In our study, glucose additions increased $P_{hex}$ by a factor of 5-8, while very little or no P was taken up when the same amount of C was added as cellulose. In the case of plant residues, the increase in microbial C, N and P after one week was closely related to the amount of $C_{H2O}$ added with crotalaria, maize stover and maize roots ($r^2 = 0.76$-$1.00$, depending on the nutrient and the soil). In an additional experiment, changes in $P_{hex}$ after addition of crotalaria and maize stover to soils COM–P and COM+P were not affected by the level of available P in the soil or by the simultaneous addition of N (Figure A1, appendix 1). This provides further evidence that the percentage of soluble C in plant residues determines the extent of microbial nutrient uptake more than levels of other soluble nutrients in the residues or in the soil. However, at high levels of soluble C as in the case of glucose additions, microbial P uptake did increase with P availability (Figure 2.3a) and was much greater when C was added together with N (chapter 1).

For longer time frames, a greater proportion of structural components will be more effective to increase the microbial biomass, as seen from the fact that at the end of experiment 2, microbial P, N (Figures 2.6 and 2.7) as well as C (data not shown) were lower in soils amended with crotalaria than with maize stover or maize roots. Also in the case of glucose, the population appeared to break down rapidly, having reached maximum P uptake after two days, resulting in re-mineralization of immobilized P (Figure 2.3a). Likewise, Vinten et al. (2002) observed greater initial changes in
microbial C after addition of glucose, whereas after 120 days, microbial C was greater in cellulose-amended soils.

Temporal dynamics of changes in $P_{hex}$ and $N_{chl}$ were similar for both soils, but in most cases, greater amounts of P and N were taken up by the microbial biomass in MCF than in COM (Figures 2.3, 2.6, and 2.7). When a subsequent release of immobilized nutrients occurred, it was also greater in MCF than in COM, as observed for changes in $P_{hex}$ after addition of glucose and crotalaria. Thus, the extent of immobilization as well as re-mineralization appears to increase with the initial size of the biomass. It may also be affected by the initial composition of the microbial community, but this cannot be distinguished from the size effect.

Microbial C:P-ratios

Changes in microbial C:P-ratios depended on the quality of plant residues, as C:P-ratios narrowed more after addition of crotalaria than of maize stover or maize roots (Figure 2.8). During re-mineralization as observed for crotalaria, C:P-ratios widened again. Temporal patterns of changes in microbial C:P-ratios were similar between soils, but the ratios were always narrower in MCF than in COM. Oehl et al. (2001a) also observed a narrowing of microbial C:P-ratios at higher levels of biomass in different farming systems. Makino et al. (2003) suggested that changes in the C:P-ratio of the bacterial biomass are caused by a shift in the community structure rather than by variations in C:P-ratios within a bacterial strain. In our study, PLFA analysis did show differences in the initial composition of the microbial biomass between MCF and COM, but too little information exists about the C:P-ratios of organism groups as indicated by PLFA to determine which groups narrowed the initial C:P-ratio by an increase in their relative abundance. More information on the relation between microbial nutrient ratios and community structure could be gained from performing PLFA analyses in incubation experiments with substrate amendments.
Changes in $P_{\text{resin}}$ and $N_{\text{K}_2\text{SO}_4}$

Changes in $P_{\text{resin}}$ were generally inversely related to changes in $P_{\text{hex}}$, except for the non-significant change in $P_{\text{resin}}$ after addition of maize stover. Similar to the increase in $P_{\text{hex}}$, the decrease in $P_{\text{resin}}$ after one week was related to the amount of $C_{\text{H}_2\text{O}}$ added with the plant residues (not shown). While microbial uptake of soil P is therefore indicated in most cases, the decrease in $P_{\text{resin}}$ generally amounted to less than the increase in $P_{\text{hex}}$. In the case of plant residues, the change in $P_{\text{hex}}$ could have been derived entirely from the amount of P added (Table 2.3, Figure 2.6). For glucose, however, a significant depletion of other P pools than $P_{\text{resin}}$ or replenishment of $P_{\text{resin}}$ from other pools must have occurred (Figures 2.3 and 2.4).

An increase in $P_{\text{resin}}$ above initial levels in the course of the experiment occurred only in the case of crotalaria, which is also the only residue for which a significant decline in $P_{\text{hex}}$ between day 7 and 183 was observed (Figure 2.6a). In a field experiment in western Kenya, a negative relationship between levels of $P_{\text{resin}}$ after one week and the $C_{\text{H}_2\text{O}}:P_{\text{tot}}$-ratio in applied plant residues was observed, suggesting that immobilization of soil P will occur at $C_{\text{H}_2\text{O}}:P_{\text{tot}} \geq 30$ (Nziguheba et al., 2000). In our study, crotalaria and maize stover induced different changes in $P_{\text{resin}}$ (as well as $P_{\text{hex}}$), although both residues had similar $C_{\text{H}_2\text{O}}:P_{\text{tot}}$-ratios. The addition of maize stover did not significantly decrease $P_{\text{resin}}$, although its $C_{\text{H}_2\text{O}}:P_{\text{tot}}$-ratio was above the threshold established by Nziguheba et al. (2000). Our results cannot be compared directly to theirs because they determined $P_{\text{resin}}$ after air-drying of soils, which may render a considerable proportion of microbial P extractable (Sparling et al., 1985). Nevertheless, our results indicate that other factors besides the ratio of $C_{\text{H}_2\text{O}}:P_{\text{tot}}$ may determine immobilization of P from soil.

The interpretation of changes in $N_{\text{K}_2\text{SO}_4}$ is limited because it represents a mixture of mineral and organic N extracted with 0.5 $M$ $K_2\text{SO}_4$. The greater increase in $N_{\text{K}_2\text{SO}_4}$ after addition of crotalaria may indicate a higher rate of N mineralization in MCF than in COM (Figure 2.7a), but it mainly shows that about 20% of $N_{\text{tot}}$ added with crotalaria was labile after only 7 days. The greater decrease in $N_{\text{K}_2\text{SO}_4}$ after addition of maize stover and maize roots in MCF than in COM is more interesting, because it reflects the difference in microbial N between soils (Figure 2.7b, c). However, $N_{\text{K}_2\text{SO}_4}$ decreased more than $N_{\text{ch}}$ increased, although 43-45 mg $N_{\text{tot}}$ kg$^{-1}$ were added with maize stover and maize roots. Unless substantial gaseous losses occurred, this points to an incomplete
extraction of microbial nutrients after fumigation, which has sometimes been corrected for by using conversion factors to calculate the amount of nutrients in the microbial biomass (e.g. Brookes et al., 1982). We did not use a conversion factor because a) it may differ between soil types and has not been determined for soils similar to the ones in our study and b) it may not be able to account for the selectivity of fumigation on groups of microorganisms (Zelles et al., 1997). Even when using a conversion factor, Vinten et al. (2002) observed that only 30% of the decrease in mineral N after cellulose addition could be explained by microbial N uptake, suggesting that a significant proportion of microbial nutrients may be missed using fumigation-extraction methods.

**Consequences for nutrient cycling in the field**

The initial immobilization increases together with soil biological activity, especially at high additions of soluble C. Due to the similarity of temporal patterns of immobilization and re-mineralization between soils, however, no measures can be suggested to optimize the synchrony between nutrient release and plant nutrient demand, except for inorganic fertilization to compensate for increased initial immobilization. Eventually, the plant may benefit from the greater re-mineralization in a soil with greater biological activity or in hot spots of soil microbial activity such as the rhizosphere.

The results from our study stress the importance to include soluble C into the characterization of plant residue quality, as suggested by Palm and Rowland (1997). As these results were obtained under controlled conditions, i.e. in the absence of plants and climatic effects, they need to be verified in the field. Different temporal patterns under field conditions may also result from the fact that in our experiment, the role of the macrofauna was simulated by the small particle size of the residues and thorough mixing with the soil.
Conclusions

In the soil from the maize-crotalaria rotation with higher initial levels of soil organic matter and microbial biomass, the apparent decomposition of all added materials proceeded faster during some parts of the incubation but differences were transient except for added crotalaria and appear to have little potential to influence nutrient cycling. However, microbial P and N uptake was in most cases related to the initial size of the microbial biomass, suggesting that the extent of microbial immobilization may increase with the biological activity of the soil. The same applies to the extent of re-mineralization from a declining biomass, as was observed for crotalaria, which may also involve an increased turnover of nutrients immobilized from the soil. Most of these effects appear to be related to the size of the microbial biomass, but changes in the composition of the microbial community may also have contributed to observed differences between soils. Compared to the effect of crop rotations, P fertilization had only a minor effect on microbial community composition and substrate use.

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An attempt to quantify rates of organic P mineralization in a highly weathered soil
“Wer misst, misst Mist”

*wisdom of a geodetic engineer*
Abstract

After an introduction to approaches used to measure the mineralization of soil organic P, an attempt to quantify the mineralization of organic P in a highly weathered soil based on isotopic dilution methods is presented, using plants as indicators. Maize was grown on $^{33}$P-labeled soils in a complete factorial design with soils from three crop rotations at two levels of P fertilization in the field and two levels of N addition in the pot experiment. Shoot dry matter, P and N content were greatest in the soil with the highest level of soil organic matter and microbial biomass and were increased by P fertilization, but differences did not amount to more than 10% deviation from the overall mean. Addition of N significantly increased maize N content by a factor of 2.7, while plant dry matter was not affected. In addition to the very low recovery of $^{33}$P in maize shoots (< 0.4%) and the absence of net P uptake into the shoot, this indicates a strong limitation of dry matter production by low P availability. The specific activities in plants growing on soils from different crop rotations did not reveal isotopic dilution of P in the soil solution due to the mineralization of organic P. Likewise, the specific activities in resin-extractable P were generally similar between crop rotations during 28 days of incubation of the same $^{33}$P-labeled soils. To quantify isotopic dilution due to organic P mineralization in resin-extractable P, a baseline of physicochemical processes would have to be established. The limitations of the approach using plants are discussed.
Chapter 3: An attempt to quantify P₀ mineralization rates

Introduction

At the low levels of inorganic P (Pᵢ) available to plants common in highly weathered soils, the mineralization of organic P (P₀) is assumed to contribute relatively more to plant P uptake than in younger soils. Path analysis of the relationship between sequentially extracted P fractions in a range of soils suggested that in slightly weathered Mollisols, resin-extractable P (P<sub>resin</sub>) was mainly replenished from Pᵢ fractions, whereas in highly weathered Ultisols, most of the variation in P<sub>resin</sub> is explained by the variation in bicarbonate-extractable P₀ (Tiessen et al., 1984). However, the direct measurement of P₀ mineralization poses difficulties because a) net changes in P₀ contents cannot be detected reliably against the large amounts of P₀ present in soils, due partly to analytical errors introduced by the indirect determination of P₀ as the difference of total P and Pᵢ in a chemically extracted fraction, and b) any Pᵢ released from P₀ may react rapidly with the soil solid phase.

For a sandy Spodosol with very little P sorption, Grierson et al. (1998) found an accumulation of Pᵢ extractable with 0.1 M KCl during 26 days of incubation, which they attributed to P₀ mineralization. The comparison of constantly moist with previously dried and rewetted soils showed that the kinetics of net P₀ mineralization changed from zero-order in constantly moist soil to a segmented model with two pools when microbial P fluctuated due to the flush effect induced by rewetting. At a constant size and activity of the microbial biomass, basal mineralization of P₀ from soil organic matter, uptake of P into the microbial biomass (immobilization) and mineralization of P₀ released during microbial death (re-mineralization) take place simultaneously. The hydrolysis of P₀ requires the activity of phosphatases, which are secreted from cells and may remain active in soils for unknown times, presumably depending on their stabilization on clays and humic colloids (Burns, 1982; Leprince and Quiquampoix, 1996). McGill and Cole (1981) suggested to differentiate between biological mineralization driven by the search for energy (C) and biochemical mineralization controlled by the need for P.

There are two different approaches to measure P₀ mineralization despite sorption of Pᵢ: the use of anion-exchange resins as an immediate sink for Pᵢ released from P₀, and the use of isotopic dilution methods. Zou et al. (1992) compared the amount of Pᵢ captured on resins during 24 h in soils sterilized by γ-irradiation (to stop microbial immobilization) without or with additional autoclaving (to denature enzymes) and in
control soils. In the controls, $P_i$ on the resins is the sum of $P_i$ desorbed from soil and $P_i$ released during $P_o$ mineralization minus $P_i$ immobilized by microorganisms, while after $\gamma$-irradiation, only immobilization is supposed to be absent, and after $\gamma$-irradiation and autoclaving, the desorption of $P_i$ from soil is the only remaining process. However, most probably $\gamma$-irradiation not only stops immobilization, but also reduces $P_o$ mineralization rates. An additional limitation of the method is that sterilization with $\gamma$-irradiation and autoclaving releases $P_i$, enzymes and other compounds from microbial cells, resulting in an overestimation of the amount of $P_i$ on the resins desorbed from the soil solid phase. The correction factors determined by Zou et al. (1992) to account for this were obtained during 1 h of extraction, although an extraction period of 24 h was chosen in the main experiment. However, according to the results of Kouno et al. (1995), hydrolysis of $P_o$ released from microbial cells continues for at least 18 h of extraction. In addition, a possible effect of sterilization on physicochemical soil properties (Wolf et al., 1989) as well as the experimental conditions of shaking 2.5 g field moist soil with 50 ml $H_2O_{dest}$ for 24 h, resembling an extraction rather than an incubation, cast some doubt on the results of Zou et al. (1992) being representative of $P_o$ mineralization in situ.

With isotopic dilution methods, isotopically exchangeable $P_i$ in the soil (soil IEP) is labeled with $^{32}P$ or $^{33}P$. Any release of unlabeled $P_i$ from unlabeled $P_o$ will then decrease the specific activity of soil IEP. Walbrigde and Vitousek (1987) and López-Hernández and Niño (1993) used this approach, determining the specific activities in acid fluoride extracts and resin-extractable $P$ ($P_{resin}$), respectively. However, the specific activity in labile $P_i$ decreases not only due to the release of unlabeled $P_i$, i.e. through biological processes, but also due to continuously ongoing isotopic exchange reactions, i.e. through physicochemical processes. Therefore, López-Hernández et al. (1998) determined the decrease in the specific activity in the soil solution in a batch experiment of isotopic exchange kinetics of 100 min duration, a time during which biological processes are assumed to be absent or minimal. The decrease in the specific activity in the soil solution for longer times (i.e. the baseline due to physicochemical processes) was then extrapolated according to Fardeau (1993) and compared to the specific activity in $P_{resin}$ of incubated soils, converting both into amounts of isotopically exchangeable $P$ (E values). From the greater E values in incubated soils, daily rates of $P_o$ mineralization of 0.2-0.9 mg P kg$^{-1}$ were derived for the Mollisols studied.
A problem which is common to all three studies is that the authors did not verify whether the specific activity in the measured pool (acid fluoride extract, $P_{resim}$) was equal to that of isotopically exchanged $P$. This was overcome by Oehl et al. (2001b) who compared the extrapolated and measured specific activities of the soil solution, i.e. measured the specific activity in the compartment into which the isotope was introduced. In order to exclude flush effects, they used $^{33}$P-labeled soils incubated at constant respiration rates. A comparison of soils from different cropping systems resulted in daily $P_0$ mineralization rates between 1.4-2.5 mg P kg$^{-1}$, the order of which was in agreement with the total content of $P_0$ as well as with soil respiration in the three soils (Oehl et al., 2003).

Soils from different crop rotations on a Ferralsol in western Kenya were found to differ in amounts of organic and microbial $P$, but not in levels of available $P_i$ (chapter 1). It is assumed that $P_0$ mineralization rates also differ between these soils. However, Oehl et al. (2001b) state that the extrapolation of specific activities from short term kinetics to longer times is not valid for soils that are low in available $P_i$ and have a high sorption capacity. For Oxisols from Colombia, Bühler et al. (2003) found the agreement between measured and extrapolated values of the decrease in $^{33}$P in the soil solution using the approach of Fardeau (1993) satisfactory, but stated that the precision of some parameters determined during the batch experiment could limit the establishment of the baseline of physicochemical reactions. In addition, a concentration of $P_i$ in the soil solution below the quantification limit of 3.6 µg l$^{-1}$ established by Bühler et al. (2003) as was observed in soils from western Kenya precludes the precise calculation of specific activities in the soil solution, especially when moist soils are used in which labile $P_i$ is even lower than in air-dried samples. Consequently, it would not be possible to convert a potential difference in the extrapolated and measured decrease in radioactivity in the soil solution into reliable $E$ values.

This chapter presents an attempt to explore if isotopic dilution due to $P_0$ mineralization occurs in soils from a field experiment in western Kenya (chapter 1), using plants as indicators of the specific activity in the soil solution. Maize was grown on $^{33}$P-labeled soils from three crop rotations, where differences in maize $P$ uptake as well as the specific activity of the plants were expected to reflect the contribution of $P_0$ mineralization to plant $P$ uptake, under the assumption that the baseline is similar for
soils from three crop rotations. This was indicated by the parameters of isotopic exchange kinetics which did not differ between crop rotations (chapter 1). Possible interactions of P\textsubscript{0} mineralization with the availability of P\textsubscript{i} and N were investigated in a complete factorial design with soils from three crop rotations at two levels of P fertilization in the field and two levels of N addition in the pot experiment. In addition, the specific activity in resin-extractable P was determined during 28 days of incubation.

**Methods**

*Experimental design*

Maize (var. H513, Kenya Seed Company) was grown for 28 days on \textsuperscript{32}P labeled soils sampled in a field experiment with three crop rotations (continuous maize (COM), maize-crotalaria fallow (MCF) and maize natural fallow (MNF) rotation) at two levels of P fertilization in the field (0 and 50 kg P ha\textsuperscript{-1} yr\textsuperscript{-1}, applied as triple superphosphate) and two levels of N addition in the pot experiment (0 and 100 mg N kg\textsuperscript{-1} soil, applied as NH\textsubscript{4}NO\textsubscript{3}). The field experiment and soil properties are described in chapter 1. A nutrient solution without P and N was added to eliminate other potential nutrient limitations. Maize was chosen as the test plant because it is the main crop in western Kenya. As the seed represents a significant source of P that may be utilized preferentially under conditions of low P availability (Truong and Pichot, 1976), seeds were removed from young seedlings before transplanting. In addition to plant P, N and label uptake, the specific activity in resin-extractable P (P\textsubscript{resin}) was determined during 28 days of incubation. Soil respiration was measured on unlabeled soils amended with the same nutrient solution, and the availability of P\textsubscript{i} was characterized with isotopic exchange kinetics on air-dried samples.

*Soil properties*

Before starting the pot experiment, equal amounts of soil from each of the four field replicates (from sampling dates 2001 and 2000 in a 4:1 ratio) were mixed to give one composite sample per treatment and incubated for 13 days at a soil moisture content of 25% (60% water holding capacity) at 25°C in the dark. Microbial P was determined as described by Kouno et al. (1995), but using hexanol as the fumigant instead of chloroform. The amount of P extracted from unfumigated subsamples is presented as P\textsubscript{resin}, while microbial P is reported as the amount of P rendered extractable by hexanol.
fumigation ($P_{hox}$) and corrected for sorption of released P during the extraction period. Total C and N ($C_{tot}$, $N_{tot}$) were determined on air-dried and ball-milled subsamples using a CN analyzer (Carlo Erba Instruments, NA 1500, Rodano-Milano, Italy).

The analysis of composite samples (Table 3.1) generally confirmed the results of the plot-wise analysis (chapter 1), except for $P_{rain}$ which showed significant effects of the cropping system and interaction with P fertilization due to high values in MNF+P.

**Preparation of maize seedlings for the pot experiment**

Maize seeds weighing between 0.60-0.65 g were surface-sterilized in 5% Ca(OCl)$_2$ for 5 min, washed in $H_2O_{dest}$ for 30 min, and pre-germinated on quartz sand in the dark for 2-3 days. They were then transferred into folded filter-papers with the tip cut off so that the roots could reach down into a box with distilled water where the seedlings were grown for 5-6 days. Before transplanting, the remainders of the maize seed were removed as completely as possible without damaging the seedling.

**Soil labeling, conditions of the pot experiment and plant analysis**

At the beginning of the experiment, the pre-incubated soils were labeled by manual stirring, adding 4 ml carrier-free $^{33}$PO$_4$ solution kg$^{-1}$ soil in order to reach a total activity of 3.7 MBq kg$^{-1}$ soil (dry weight equivalent) without significant alteration of the soil water content. At the same time, a P-free nutrient solution was added at 5 ml kg$^{-1}$ soil, which supplied 80 mg K, 17 mg S, 12 mg Mg, 2 mg Cu, 2 mg Mn, 1 mg Zn, 1 mg B and 0.1 mg Mo kg$^{-1}$ soil, and 100 mg N kg$^{-1}$ in the case of +N treatments only. Ca was not added in order to avoid any interference with the P sorption behavior of the soils, as proposed by Abekoe and Tiessen (1998). The exchangeable Ca content of the soils of about 3 cmol$_c$ kg$^{-1}$ was considered sufficient to meet the Ca demand of the maize plants. Two days after addition of the nutrient solution, soil pH was not significantly changed in –N treatments, while in +N treatments, it had decreased by 0.15 units (not shown).

Labeled moist soil equal to 500 g dry soil was filled into four pots per treatment. Two maize seedlings were planted in each pot. At transplanting, the majority of seedlings had developed a short compact root system, while some had developed very long roots, probably depending on the size and position of the young roots placed on the filter paper. As not enough healthy-looking seedlings of one type of root system were available, repetitions 1 and 2 were entirely planted with seedlings of the short-root type.
while in repetitions 3 and 4 one plant had short and the other long roots, the position of which was noted in order to include this factor in the statistical analysis.

The conditions in the climate chamber were: a day/night cycle of 14/10 h with 25/20°C, 70% relative humidity, and a light intensity at the soil surface of 300-400 μmol s⁻¹ m⁻². The position of the pots was changed repetition-wise daily, when water losses from the pots were replaced by weight. After 28 days, plants were cut at the soil surface and weighed fresh as well as after drying for 5 days at 60°C. Plant material (0.3-0.5 g) was cut into pieces of 5-10 mm length and ashed at 550°C for 8-10 h. The ash was dissolved in 2 ml 20% HCl, the solution was heated shortly, filtered (Whatman no. 40) with addition of H₂O₉est and made to 50 ml volume. The concentration of P was determined colorimetrically (Murphy and Riley, 1962). The radioactivity in the filtrate was determined using a liquid scintillation counter (Packard 2500 TR) with 5 ml Packard Ultima Gold scintillation liquid per 1 ml of sample (diluted with H₂O₉est in the case of

### Table 3.1: Selected properties of the composite soils used in the pot experiment \( (n = 4) \) and soil respiration during 28 days of soils amended with a nutrient solution adding N at 0 (−N) and 100 (+N) mg N kg⁻¹, respectively \( (n = 3) \)

<table>
<thead>
<tr>
<th>field treatment</th>
<th>( \text{P}_{\text{resin}}^{a} ) mg kg⁻¹</th>
<th>( \text{P}_{\text{hex}} ) g kg⁻¹</th>
<th>( \text{C}_{\text{tot}} ) g kg⁻¹</th>
<th>( \text{N}_{\text{tot}} ) g kg⁻¹</th>
<th>cumulative CO₂-release ( ^{b} ) mg C kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM–P</td>
<td>2.2 c 2.5 c</td>
<td>22.5 c 1.5 bc</td>
<td>55.2 c 72.2 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF–P</td>
<td>1.6 c 5.2 ab</td>
<td>25.4 ab 1.9 a</td>
<td>133.7 b 136.3 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNF–P</td>
<td>1.8 c 4.0 b</td>
<td>23.7 bc 1.7 ab</td>
<td>155.3 ab 145.6 ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COM+P</td>
<td>9.5 b 2.3 c</td>
<td>22.3 c 1.4 c</td>
<td>73.2 c 86.7 c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCF+P</td>
<td>8.9 b 6.2 a</td>
<td>26.4 a 1.9 a</td>
<td>164.6 a 166.5 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNF+P</td>
<td>10.8 a 4.8 ab</td>
<td>24.7 ab 1.6 abc</td>
<td>158.2 ab 147.3 ab</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source of variation

- rotation (R) *** *** *** *** *** ***
- P fert. (P) *** ns ns ns * **
- R x P *** ns ns ns ns ns

* \( P = 0.01-0.05 \), ** \( P = 0.01-0.001 \), *** \( P < 0.001 \), ns = not significant

Within columns, means followed by the same letter are not significantly different \( (P = 0.05) \) by Tukey’s multiple range test

* extraction period of 16 h

\( ^{b} \) the effect of N addition on soil respiration was not significant
acid extracts) and corrected for decay to the time of soil labeling. The remaining plant material was ball-milled for the determination of N concentrations as described above.

**Specific activity of resin-extractable P**

Parallel to the pot experiment, labeled soils equivalent to 100 g dry weight were incubated in perforated polyethylene bags in a dark box in the climate chamber. On day 4, 9, 15, 22, and 28 after soil labeling, subsamples were removed for the determination of the specific activity in $P_{\text{resin}}$. Briefly, moist soil equivalent to 2 g dry weight ($n = 4$) was shaken horizontally with 30 ml $H_2O_{\text{dest}}$ and 2 resin membranes for 16 h at 170 reciprocations min$^{-1}$. The resin membranes were then rinsed carefully with $H_2O_{\text{dest}}$ and eluted with 20 ml 0.5 M HCl. The amount of P and the radioactivity in the eluate were determined as described above and corrected for decay to the time of soil labeling.

**Analyses performed on unlabeled, nutrient-amended, unplanted soils**

For each field treatment, a pre-incubated composite sample of about 200 g of unlabeled soil was amended with nutrient solution in the same proportion as the labeled soils. The CO$_2$ released from incubated soils (20 g, $n = 3$) was trapped in NaOH and determined at the end of the pot experiment by titration with HCl (Alef, 1995). Another part of the unlabeled soils was incubated in a dark box in the climate chamber for two days after addition of the nutrient solution. Soils were then dried at 45°C for 48 h and characterized for $P_i$ availability by isotopic exchange kinetics ($n = 3$) as described by Bühler et al. (2003). The ratio between the radioactivity remaining in the solution after one minute of exchange ($r_1$) and the total introduced radioactivity ($R$) is correlated to the P sorption capacity (Tran et al., 1988) and the parameter $n$ represents the rate of disappearance of radioactivity from the solution for exchange times longer than one minute. The pool of free ions which are immediately plant available is approximated by the amount of P exchangeable within 1 min ($E_{1\text{min}}$) in mg P kg$^{-1}$ calculated as

$$E_{1\text{min}} = 10*cp*R/r_1$$

(3)

where $cp$ is the concentration of $P_i$ in the soil solution and the factor 10 results from the soil:solution ratio of 1:10.
Statistical analysis
Data from the pot experiment were analyzed in SYSTAT (SPSS 2000) with ANOVA including the factors crop rotation (R), P fertilization (P), N addition (N), root length and repetition and the interactions R x P, R x N, P x N and R x P x N. Results from soil analyses were analyzed with ANOVA with the factors R, P, N, and all two- and three-way interactions. Significant effects were tested with Tukey’s multiple range test (P ≤ 0.05).

Results and Discussion

Soil respiration
The cumulative CO₂-release from soils during 28 days was higher in MNF and MCF than in COM, and it was also increased by P fertilization (Table 3.1). The addition of N did not significantly affect soil respiration. Cumulative soil respiration measured plot-wise on freshly sampled soils in the previous year was almost twice as high (chapter 1), possibly due to the slightly higher water content (30%) and the absence of a pre-incubation period. Alternatively, microbial activity in the soils used in the pot experiment may have been reduced during storage of moist soils at 4°C, which amounted to 2 and 14 months for the soils sampled in 2001 and 2000, respectively.

Plant growth, dry matter production, nutrient and label uptake
Three days after transplanting, all plants had survived the removal of the seed, looked green and healthy, and had begun to elongate. After 20 days, however, most plants had developed a purple leaf color and older leaves had become senescent. At harvest (after 28 days), even some of the younger leaves began to die back from the tip.

Above-ground plant biomass ranged in the order MCF ≥ MNF ≥ COM (Table 3.2). The concentrations of P and N in the plant biomass did not differ among crop rotations, and differences in P and N uptake reflected those in plant dry matter production. P fertilization significantly increased plant dry matter, P concentrations and P uptake, whereas it decreased N concentrations and had no effect on N uptake. Addition of N did not affect plant dry matter, but increased P concentrations and P uptake. While all differences reported so far were within ±10% deviation from the overall mean, plant N concentrations and uptake more than doubled after N addition.
### Table 3.2: Shoot dry matter, P and N concentration and content after 28 days of growth

<table>
<thead>
<tr>
<th>factor</th>
<th>dry matter g shoot&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P conc. mg P g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>P content mg P shoot&lt;sup&gt;1&lt;/sup&gt;</th>
<th>N conc. mg N g&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>N content mg N shoot&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crop rotation (R)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COM</td>
<td>0.80 b</td>
<td>0.61 ns</td>
<td>0.48 b</td>
<td>13.8 ns</td>
<td>11.0 b</td>
</tr>
<tr>
<td>MCF</td>
<td>0.90 a</td>
<td>0.61 ns</td>
<td>0.56 a</td>
<td>14.9 ns</td>
<td>13.3 a</td>
</tr>
<tr>
<td>MNF</td>
<td>0.88 ab</td>
<td>0.62 ns</td>
<td>0.54 ab</td>
<td>14.1 ns</td>
<td>12.1 ab</td>
</tr>
<tr>
<td><strong>P fertilization (P)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>minus</td>
<td>0.82 b</td>
<td>0.59 b</td>
<td>0.48 b</td>
<td>14.7 a</td>
<td>11.9 ns</td>
</tr>
<tr>
<td>plus</td>
<td>0.89 a</td>
<td>0.64 a</td>
<td>0.57 a</td>
<td>13.8 b</td>
<td>12.4 ns</td>
</tr>
<tr>
<td><strong>N addition (N)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>minus</td>
<td>0.84 ns</td>
<td>0.58 b</td>
<td>0.49 b</td>
<td>7.8 b</td>
<td>6.6 b</td>
</tr>
<tr>
<td>plus</td>
<td>0.87 ns</td>
<td>0.65 a</td>
<td>0.57 a</td>
<td>20.7 a</td>
<td>17.7 a</td>
</tr>
</tbody>
</table>

Within columns and factors, means followed by the same letter are not significantly different ($P = 0.05$) by Tukey’s multiple range test; ns: not significant; two- and three-way interactions between the factors R, P and N were not significant.

The sufficiency range of N and P concentrations in maize plants at the 3- to 4-leaf stage is given as 35-50 mg N g<sup>-1</sup> and 4-8 mg P g<sup>-1</sup> (Bennett, 1993). While such values differ among cultivars, it can be stated that the concentrations in the maize shoots were far below these levels in all treatments of the pot experiment, especially for P. It appears that the plants were primarily P limited, since in +N treatments, more N was taken up by the plants without resulting in a higher dry matter production. However, the fact that P uptake was increased by addition of N points to an interaction or a co-limitation.

The average P content of 0.53 mg P shoot<sup>1</sup> contrasts with the P content of whole maize seeds of 1.71 mg P (SD ± 0.22, $n = 18$) and the mean P content of seedlings after seed removal of 1.17 mg P (SD ± 0.11, $n = 18$). On average, 72% of seedling P (0.85 mg) was located in the shoot. This suggests that in the pot experiment, no net uptake into the shoot was detected, and a net P transfer into the roots must have occurred. Under P deficiency, a net translocation of P from the shoot to the root has been reported, at least for a forage legume (Smith et al., 1990).
Table 3.3: Percentage of applied label and relative specific activities (SA) in maize shoots, and parameters of isotopic exchange kinetics of the soils from the pot experiment (n = 3)

<table>
<thead>
<tr>
<th>field treatment</th>
<th>label shoot(^{-1}) % of applied (^{33})P</th>
<th>rel. SA % of label mg P(^{-1})</th>
<th>r1/R</th>
<th>n</th>
<th>cp(^a)</th>
<th>E(_{1\text{min}})(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM–P</td>
<td>0.08 d</td>
<td>0.16 d</td>
<td>0.016 bc</td>
<td>0.569 a</td>
<td>0.002 ns</td>
<td>1.0 ns</td>
</tr>
<tr>
<td>MCF–P</td>
<td>0.05 d</td>
<td>0.09 d</td>
<td>0.015 bc</td>
<td>0.594 a</td>
<td>0.001 ns</td>
<td>0.8 ns</td>
</tr>
<tr>
<td>MNF–P</td>
<td>0.07 d</td>
<td>0.14 d</td>
<td>0.013 c</td>
<td>0.573 a</td>
<td>0.001 ns</td>
<td>0.8 ns</td>
</tr>
<tr>
<td>COM+P</td>
<td>0.13 c</td>
<td>0.28 c</td>
<td>0.020 ab</td>
<td>0.536 b</td>
<td>0.003 ns</td>
<td>1.5 ns</td>
</tr>
<tr>
<td>MCF+P</td>
<td>0.21 b</td>
<td>0.36 b</td>
<td>0.022 a</td>
<td>0.515 bc</td>
<td>0.002 ns</td>
<td>1.0 ns</td>
</tr>
<tr>
<td>MNF+P</td>
<td>0.33 a</td>
<td>0.55 a</td>
<td>0.022 a</td>
<td>0.498 c</td>
<td>0.003 ns</td>
<td>1.4 ns</td>
</tr>
<tr>
<td>N addition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–N</td>
<td>0.13 b</td>
<td>0.25 ns</td>
<td>0.019 a</td>
<td>0.548 ns</td>
<td>0.002 ns</td>
<td>1.2 ns</td>
</tr>
<tr>
<td>+N</td>
<td>0.16 a</td>
<td>0.27 ns</td>
<td>0.017 b</td>
<td>0.547 ns</td>
<td>0.002 ns</td>
<td>1.0 ns</td>
</tr>
</tbody>
</table>

Within columns and factors, means followed by the same letter are not significantly different (P = 0.05) by Tukey’s multiple range test; ns: not significant; interactions between R*N, P*N and R*P*N were not significant.

\(^{a}\) significant effect of P fertilization (–P: 0.001, +P: 0.003), but the interaction R*P was not significant.

\(^{b}\) significant effect of P fertilization (–P: 0.9, +P: 1.3), but the interaction R*P was not significant.

Nevertheless, a small percentage of label was recovered in the shoots of maize, not surpassing 0.4% for any of the treatments (Table 3.3). Both P fertilization and N addition increased the recovery of label in the maize shoot (P = 0.000 and 0.009, respectively). The recovery of \(^{33}\)P and resulting specific activities (\(^{33}\)P/\(^{31}\)P) in maize shoots also showed a significant interaction between crop rotation and P fertilization. For the –P treatments, label uptake and specific activities did not differ among crop rotations, whereas within the +P treatments, they ranged in the order COM < MCF < MNF.
Chapter 3: An attempt to quantify P₀ mineralization rates

Figure 3.1: Decrease in the specific activity in \( P_{\text{resin}} \) of the soils used in the pot experiment during 28 days of incubation

Specific activity in \( P_{\text{resin}} \)

On all dates, the specific activity in \( P_{\text{resin}} \) was significantly higher in –P than in +P treatments but was not affected by N addition and did not differ among crop rotations, except on days 4 and 9 when it was higher in MCF than in MNF and COM (Figure 3.1).

Isotopic exchange kinetics performed on unlabeled, unplanted soils

Values of \( r_1/R \) were increased by P fertilization and decreased by N addition (Table 3.3), possibly as a result of the decrease in pH observed in the +N treatments. The parameter \( n \) was significantly lower in +P than in –P treatments, without being affected by N addition. Crop rotation generally had no effect on these two parameters. In spite of high coefficients of variation for the concentration of \( P_i \) in the soil solution (cp) of up to 100% \( (n = 3) \), P fertilization significantly increased cp and values of \( E_{1\text{min}} \).
Relationship between plant P uptake and P, availability

Averaged across levels of P fertilization and N addition, the difference in P contained in the above-ground biomass of maize growing on MCF and COM, respectively, amounts to 0.08 mg P per shoot (Table 3.2). This translates into 0.32 mg P kg⁻¹ soil, as two plants were grown in 0.5 kg soil. This difference may reflect a different rate of basal mineralization of P₀ from soil organic matter in these soils, but this cannot be verified, especially in view of the absence of net P uptake into the shoot.

In the absence of a reliable baseline for isotopic dilution in the soil solution due to physicochemical processes, the specific activities in plant tissue can only be compared under the assumption that soils from the three crop rotations did not differ in the availability of P₁. This was generally confirmed, except for the significant interaction between MNF and P fertilization observed for P_resin (Table 3.1). The similar specific activities in maize shoots for the three crop rotations in the -P treatments (Table 3.3) do not give an indication that the additional P content in maize shoots on MCF compared to COM was derived from mineralized P₀. In the +P treatments, the order of specific activities in maize shoots of COM < MCF < MNF also contradicts the initial hypothesis that the specific activity would be highest in COM where the lowest P₀ mineralization is expected. Instead, the high specific activity in MNF+P may reflect the high value of P_resin in the composite soil (Table 3.1), although E₁imin did not differ from the other crop rotations (Table 3.3).

Also the specific activity in P_resin did not differ between crop rotations (Figure 3.1). Under the assumption of a similar baseline of physicochemical processes in soils from the three rotations, this would indicate that isotopic dilution in P_resin due to P₀ mineralization was not detected or occurred to a similar extent in soils from the three rotations. In order to quantify P₀ mineralization rates by comparing extrapolated and measured specific activities in a pool different from the soil solution, the baseline should be established during a short-term batch experiment for the respective pool. A preliminary test if this would be possible for P_resin is presented in Appendix 2.
Conclusions

The pot experiment indicated small differences in plant dry matter and nutrient uptake for the soils from the three crop rotations, but was not successful in that no net P uptake into the shoot was detected. Under these circumstances, plants cannot be used to detect isotopic dilution due to the mineralization of soil P. An additional limitation of the use of plants as indicators for the availability of a single nutrient lies in the fact that nutrient uptake is affected by all factors influencing plant growth, as observed for increased P uptake after addition of N in this experiment.

Label recovery in maize shoots did reflect differences in P availability due to P fertilization in the field. This suggests that the tested approach to grow maize after removal of the seed as an unknown source of P could be used to investigate the availability of freshly added sources of P labeled with $^{33}$P. However, a good estimate of the P content of a seedling at transplanting and the harvest of the shoot as well as the root are required to quantify net P uptake.
Microbial and plant uptake of P derived from the soil, from added plant residues or from added inorganic P in a highly weathered soil
„The role of the infinitely small is infinitely large“

*Louis Pasteur*
Abstract

In order to elucidate microbial processes in P dynamics in a highly weathered soil, and their effects on P availability to plants, soils from two crop rotations (continuous maize and maize-crotalaria rotation) from a field experiment on a Ferralsol in western Kenya were incubated for nine weeks after addition of inorganic P (P_i) or a plant residue, both labeled with $^{33}$P and added at 6 mg P kg$^{-1}$, or after carrier-free labeling of isotopically exchangeable P_i (soil IEP). The amount of P and recovery of $^{33}$P was determined in microbial P, resin-extractable P (P_resin) and in a sequential extraction of fumigated samples with 0.1 M NaOH, and maize was grown on the same soils in a parallel pot experiment. Up to 18% of applied $^{33}$P was recovered in microbial P, ranging in the order plant residue > soil IEP ≥ added P_i. More than half of the increase in microbial P after residue amendment was derived from soil P. Only P_i addition increased P_resin, where the recovery of $^{33}$P followed the order added P_i > soil IEP > residue. The recovery of $^{33}$P in P_resin decreased steadily from 7-22% after one day to 3-5% after nine weeks, while it remained constant in microbial P. The higher microbial activity in soil from the maize-crotalaria rotation increased the recovery of $^{33}$P in microbial P and decreased that in P_resin compared to soil from continuous maize. In the NaOH extract, an additional 66-76% of $^{33}$P was recovered, with the highest recovery after plant residue addition. In residue-amended soils, up to 27% of $^{33}$P in the NaOH extract was recovered in organic P and up to 8% in the treatments soil IEP and addition of P_i. During growth periods of three weeks, maize took up a maximum of 2% of $^{33}$P from added P_i, compared to 0.1-0.5% from the plant residue and after labeling of soil IEP, but the interpretation was limited by the absence of net P uptake by maize. While the importance of the microbial biomass in P dynamics was suggested by its rapid initial uptake of P, subsequent fluxes in and out of microbial P could not be measured because of the fast removal of $^{33}$P from P_resin due to physicochemical reactions.
Chapter 4: Microbial and plant uptake of P from different sources

Introduction

On highly weathered, P-limited soils in western Kenya, higher maize yields and P uptake after a one-season fallow with legumes such as Crotalaria grahamiana Wight & Arn. (subsequently referred to as crotalaria) than after maize (Niang et al., 2002; Smestad et al., 2002, and chapter 1 of this thesis) suggest improved P availability after incorporation of fallow biomass. Higher levels of organic and microbial P are observed in the topsoil under maize-fallow rotations than under continuous maize, while the availability of inorganic P (Pi) does not differ between crop rotations (Smestad et al., 2002 and chapter 1 of this thesis). The relative contribution of soil biological processes to plant P uptake may increase when the availability of Pi is low.

In an incubation experiment with soils from western Kenya, addition of crotalaria residues increased microbial P by 250% within the first week, while it decreased resin-extractable P (Presm) to 70-80% of initial values (chapter 2). The absolute decrease in Presm amounted to less than 10% of the increase in microbial P, but in the absence of isotopic labeling, the contribution of plant residue and soil P to microbial P uptake could not be determined. The same applied to the comparison of soils from the maize-crotalaria rotation and continuous maize, where the extent of microbial P uptake after addition of plant residues increased with the initial amount of microbial biomass.

The source of microbial P can be determined by combining fumigation-extraction methods with isotopic labeling, similar to isotopic techniques used in combination with analysis of other soil P pools and plants (Fardeau et al., 1996). With carrier-free labeling of isotopically exchangeable soil P (soil IEP), the number and/or sizes of pools in a complex system and their kinetic relations are determined, while the effectiveness of a P fertilizer is investigated by adding a labeled amount of P. McLaughlin and Alston (1986) applied 33P-labeled medic (Medicago trunculata) residues and 32P-labeled calcium phosphate in a pot experiment, either alone or in combination. After 34 days, 65 and 29% of simultaneously added 33P and 32P, respectively, were recovered in the microbial biomass, compared to 10% and 8% in the above-ground wheat biomass. These results point to the importance of the microbial biomass in soil P dynamics.

In order to elucidate P dynamics in a highly weathered soil, we followed the distribution of three P sources labeled with 33P (carrier-free labeled soil IEP, added Pi and plant residue P) into different P pools of soils from two crop rotations (continuous maize and
maize-crotalaria rotation) of a field experiment on a Ferralsol in western Kenya. While the main focus was on the extent and temporal dynamics of microbial P uptake during nine weeks of incubation, the amount of P and recovery of $^{33}$P in $P_{\text{resin}}$ and in a sequential extraction of fumigated samples with 0.1 M NaOH were also investigated. In a parallel pot experiment, maize was planted on the same soils after different times of incubation and grown for three weeks each, aiming at a resolution of temporal patterns of immobilization and re-mineralization.

**Methods**

*Experimental design*

The experiment had a two-factorial design, with three different P sources (carrier-free labeling of soil IEP, addition of labeled P, and of a labeled plant residue) and soils from two crop rotations (continuous maize and maize-crotalaria rotation). Figure 4.1 gives an overview of the time scale of the experiment. After soil amendment on day 0, portions of soil were incubated for the determination of microbial and resin-extractable P, sequentially extracted P and soil respiration. Pots for each growth phase were filled and planted to maize either directly or after incubation for 21 and 42 days, respectively.

<table>
<thead>
<tr>
<th>days after amendment</th>
<th>1</th>
<th>10</th>
<th>21</th>
<th>30</th>
<th>42</th>
<th>51</th>
<th>63</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>sequ. P fract.</td>
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<tr>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>growth phase 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.1: Time scale of the experiment

A similar incubation experiment but without radioactive labeling was run ahead of the main experiment to determine changes in microbial P ($P_{\text{hex}}$), which was required for the sorption correction for the recovery of $^{33}$P in $P_{\text{hex}}$. The unlabeled soils were also used for the determination of microbial C and N and soil pH on day 21, 42 and 63 after amendment.
**Soil sampling and soil properties**

Soil samples were taken in January 2002 in a field experiment in western Kenya (0°09' N, 34°33' E) on a kaolinitic, isohyperthermic Kandiudalfic Eutrudox (USDA classification) or a Ferralsol (FAO) with 39% clay and 37% sand in the top 15 cm. In this experiment, different crop rotations (continuous maize and various maize-fallow rotations) and P fertilization rates have been compared since 1997 in a randomized block design with four replications. For the present study, two crop rotations were selected which did not receive any inorganic P fertilization. Continuous maize (COM) represents the traditional system with two maize crops per year, whereas the rotation of maize with a crotalaria fallow (MCF) is tested as an option for soil fertility improvement (Table 4.1). Further details of the field experiment are given in chapter 1.

Composite soil samples consisting of 15 random cores were collected from each plot (0-15 cm). At this time, maize had just been harvested while the fallows of the fifth fallow phase were still standing. Field-moist samples were sieved at 4 mm to remove coarse plant debris, and stored at 4°C until the set up of the experiment. A small portion of each sample was air-dried and sieved at 2 mm before chemical analysis. The selected field treatments did not significantly affect soil pH (4.9), CEC (6.4 cmol c kg⁻¹) and base saturation (64%). Soil organic C and N as well as microbial C, N and P were significantly higher in MCF while $P_{\text{resin}}$ was lower (Table 4.1).

### Table 4.1: Crop rotations selected for the study and effect on organic, inorganic and microbial nutrients in the 0-15 cm soil layer

<table>
<thead>
<tr>
<th>rotation</th>
<th>crop in season</th>
<th>C$_{\text{tot}}$</th>
<th>N$_{\text{tot}}$</th>
<th>P$_{\text{o}}$</th>
<th>N$_{\text{min}}$</th>
<th>P$_{\text{resin}}$</th>
<th>C$_{\text{chl}}$</th>
<th>N$_{\text{chl}}$</th>
<th>P$_{\text{hex}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>COM</td>
<td>maize</td>
<td>23.0</td>
<td>1.6</td>
<td>302</td>
<td>11.7</td>
<td>1.2</td>
<td>90</td>
<td>13</td>
<td>2.9</td>
</tr>
<tr>
<td>MCF</td>
<td>maize</td>
<td>26.0</td>
<td>1.9</td>
<td>335</td>
<td>7.7</td>
<td>0.9</td>
<td>152</td>
<td>21</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* P = 0.01-0.05, ** P = 0.01-0.001, *** P < 0.001, ns = not significant

$^a$ determined on each of the four field replications
$^b$ total C and N by dry combustion
$^c$ organic P by ignition (Saunders and Williams, 1955)
$^d$ mineral N extracted with 2 M KCl
$^e$ P extractable with anion-exchange resin membranes
$^f$ microbial C and N by fumigation-extraction (Vance et al., 1987)
$^g$ microbial P by fumigation-extraction (Kouno et al., 1995)
Chapter 4: Microbial and plant uptake of P from different sources

Production of $^{33}$P labeled crotalaria residues

Pre-soaked crotalaria seeds (provenance: Maseno, Kenya) that had germinated on filter paper at 33°C in the dark were transferred to a box with 22 l of a 25% nutrient solution after Hoagland and Arnon (1938) but without P, which was placed in a climate chamber (day/night 28/18°C, 14/10h, 65% relative humidity, light intensity approx. 500 µmol s$^{-1}$ m$^{-2}$). Six days after germination, 48 seedlings were transferred to another box with aerated 25% P-free Hoagland solution, where they received a total of 68.1 mg P and 185 MBq applied with a $^{33}$P labeled $1M$ KH$_2$PO$_4$ solution and were grown for 22 days. At harvest, the shoots were cut and the roots washed twice in H$_2$O$_{dest}$ before drying for 48h at 70°C. Shoots and roots were crushed together through a 1 mm sieve. The stalks remaining on the sieve (approx. 8 g) were ball-milled for 1 min and pooled with the crushed parts.

Ahead of the $^{33}$P labeled crotalaria residues, unlabeled residues had been produced in the same way, ball-milled subsamples of which were used for the determination of total C and N with a CN analyzer (Carlo Erba Instruments, NA 1500, Rodano-Milano, Italy) and the concentration of K, S, Ca, Mg, Cl and micronutrients with an X-ray fluorescence spectrometer (Spectro X-Lab2000, Kleve, Germany).

The concentration of total P (P$_{tot}$) in both residues and the radioactivity in the labeled ones were determined after dry combustion at 550°C and dissolution of the ash in 2 ml 20% HCl ($n = 6$). The amount of P and radioactivity extracted by anion-exchange resin membranes (BDH #55164, 31 mm x 20 mm) were determined in the presence and absence of 1 ml hexanol, respectively ($n = 4$). The concentration of P$_i$ in all extracts was determined colorimetrically (Murphy and Riley, 1962), while the radioactivity was determined using a liquid scintillation counter (Packard 2500 TR) with 5 ml Packard Ultima Gold scintillation liquid per 1 ml of sample. In order to achieve complete recovery of standard additions of $^{33}$P, dilution with H$_2$O$_{dest}$ or neutralization with NaOH was required in the case of acid extracts.

For unknown reasons, dry matter production and P concentration differed between labeled and unlabeled residues (Table 4.2), but resulted in a similar P uptake of 76 mg P. Subtracting the 0.18 (SD ±0.02) mg P per seed ($n = 5$), 99% of the added P was recovered. The P concentration of the residues was similar and the N concentration somewhat higher than that of crotalaria leaves sampled in the field experiment (chapter
Table 4.2: Total dry matter, C, N, and P concentration, and specific activity of total and resin-extractable P in $^{33}$P-labeled and unlabeled crotalaria residues

<table>
<thead>
<tr>
<th>Crotalaria residue</th>
<th>Dry matter $^a$</th>
<th>$C_{\text{tot}}$</th>
<th>$N_{\text{tot}}$</th>
<th>$P_{\text{tot}}$</th>
<th>$P_{\text{resin}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g DM</td>
<td>mg g$^{-1}$ DM</td>
<td>kBq mg$^{-1}$</td>
<td>mg P g$^{-1}$ DM</td>
<td>kBq mg$^{-1}$ $^{31}$P$^b$</td>
</tr>
<tr>
<td>Labeled</td>
<td>42.1</td>
<td>nd</td>
<td>nd</td>
<td>1.81 a</td>
<td>1.25 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1135</td>
<td>1.27 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>1424</td>
<td>1435</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unlabeled</td>
<td>48.9</td>
<td>437</td>
<td>30.0</td>
<td>1.56 b</td>
<td>0.93 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>na</td>
<td>0.99 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

Within columns, means followed by the same letter do not significantly differ between labeled and unlabeled residues; nd = not determined, na = not applicable; ns = no significant difference between fumigated and non-fumigated samples; all analyses performed with 4-6 analytical replications.

$a$ total dry matter of 48 plants

$b$ corrected for radioactive decay to the start of the experiment (time of amending soil)

$c$ nf = absence, f = presence of hexanol during $P_{\text{resin}}$ extraction, respectively

1. For the labeled residue, the specific activity ($^{33}$P/$^{31}$P) was higher in $P_{\text{resin}}$ than in $P_{\text{tot}}$, suggesting that homogenous labeling was not achieved. Neither the P concentration nor the specific activity in $P_{\text{resin}}$ was affected by the presence of hexanol during extraction. Thus, the addition of the plant residue did not represent a source of error for the determination of microbial P by hexanol fumigation of soils.

Soil amendment and conditions of incubation

Before the experiment, equal amounts of soil from each of the four field replications of the same rotation were thoroughly mixed to give a composite sample and preconditioned at 25% water content (60% water holding capacity) at 25°C for 12 days in the dark. At the start of the experiment, soils were labeled and amended with the different P sources (Table 4.3) in portions of 2.1 kg soil, which were stirred manually for 15 min. The amount of plant residue added (3.3 g DM kg$^{-1}$ soil) corresponds to a biomass of 5 Mg DM ha$^{-1}$ incorporated into the 0-15 cm soil layer which is the common tillage depth in western Kenya. The addition of $P_{1}$ was adjusted so as to add the same amount of P as with the plant residue. The non-residue amended treatments received a nutrient solution containing the nutrients added with the crotalaria residue. As only a part of the total N ($N_{\text{tot}}$) of the plant residue was expected to be mineralized during the
Table 4.3: Description of the factor ‘labeled P source’

<table>
<thead>
<tr>
<th>labeled P source</th>
<th>description</th>
<th>P added mg P kg⁻¹ soil</th>
<th>applied radioactivity¹ kBq kg⁻¹ soil</th>
<th>other nutrients applied with</th>
<th>nutrient solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>soil IEP&lt;sup&gt;c&lt;/sup&gt;</td>
<td>carrier-free labeling of soil IEP with $^{33}$P</td>
<td>0</td>
<td>10332</td>
<td>nutrient solution</td>
<td></td>
</tr>
<tr>
<td>added P&lt;sub&gt;i&lt;/sub&gt;</td>
<td>addition of $^{33}$P labeled phosphoric acid</td>
<td>6</td>
<td>7533</td>
<td>nutrient solution</td>
<td></td>
</tr>
<tr>
<td>plant residue</td>
<td>addition of $^{33}$P labeled crotalaria residue (3.3 g DM kg⁻¹ soil)</td>
<td>6</td>
<td>6757</td>
<td>plant residues</td>
<td></td>
</tr>
</tbody>
</table>

¹ corrected for radioactive decay to the time of soil amendment

² added in mg kg⁻¹ soil: 49.5 and 99.0 N (nutrient solution and plant residues, respectively); 71.7 K, 6.3 S, 40.2 Ca, 9.8 Mg, 17.5 Cl, 0.1 Mn, 0.07 Cu, 0.3 Zn, 0.01 Mo, and 0.25 Bo

<sup>c</sup> soil IEP: isotopically exchangeable soil P

experiment, half the amount of $N_{tot}$ added with the residue was applied to the non-residue amended treatments (as NH₄⁺ and NO₃⁻ in a ratio of 1:4). For the other nutrients, the amounts added with the nutrient solution equaled those added with the residue.

For the respiration measurement, portions of 20 g (dry weight) were incubated in closed bottles containing an NaOH trap, while for microbial extractions, 300 g soil per treatment was filled in a 1 l plastic container with a perforated lid, subsamples of which were removed at each sampling date. For the pot experiment, 18 wide-mouth plastic bottles (500 ml) per treatment were filled with 325 g soil each, 6 of which were used for each growth phase. The unplanted pots were incubated together with the soils for the respiration measurement and microbial extractions in dark boxes, which were placed in the climate chamber where the pot trial was conducted under the same conditions as for the production of crotalaria residues. Water losses from incubated samples were adjusted gravimetrically every week.
General calculations for the recovery of $^{33}$P

As the total applied radioactivity differed between the P sources (Table 4.3), the recovery of $^{33}$P in a given pool (microbial P, $P_{\text{resin}}$, NaOH-P, maize) is calculated as

$$\text{recovery} \, (\%) = \left( \frac{r}{R} \right) \times 100$$

where $r$ and $R$ are the radioactivity recovered in the pool and the total applied radioactivity, respectively (Fardeau et al., 1996). Likewise, specific activities ($^{33}$P/$^{31}$P) are presented as relative specific activities (SA) according to

$$SA = \left( \frac{r}{R} \right) / QP$$

where $QP$ is the amount of P (in mg P kg$^{-1}$) in a given pool. The proportion of a given pool derived from a labeled P addition ($P_{\text{dff}}$), i.e. from added $P_i$ or plant residue P, is calculated as

$$P_{\text{dff}} \, (\%) = \left( \frac{SA_{\text{pool}}}{SA_{\text{add}}} \right) \times 100$$

where $SA_{\text{pool}}$ and $SA_{\text{add}}$ are the specific activities of the pool and the labeled P addition, respectively. The amount of P derived from a given P source ($q$) is calculated as

$$q \, (\text{mg P kg}^{-1}) = \left( \frac{SA_{\text{pool}}}{SA_{\text{add}}} \right) \times QP$$

In case of a net increase in $QP$ after a labeled P addition over the treatment soil IEP, the additional amount of P derived from soil ($s$) is

$$s \, (\text{mg P kg}^{-1}) = QP_{\text{add}} - QP_{\text{soilIEP}} - q$$

where $QP_{\text{add}}$ is the amount of P in a given pool after addition of $P_i$ or plant residue and $QP_{\text{soilIEP}}$ that in the treatment soil IEP.

As soil IEP was labeled carrier-free, in this treatment no specific activity was available to calculate $P_{\text{dff}}$ and $q$.

Soil respiration, microbial C and N, and soil pH

Soil respiration ($n = 3$) was determined by trapping CO$_2$ in 0.05-0.2 M NaOH, as required depending on the P source and measurement interval, and titration with 0.1-0.4 M HCl (Alef, 1995). C and N held in the microbial biomass ($n = 3$) were determined by 24 h fumigation followed by extraction with 0.5 M K$_2$SO$_4$ as described by Vance et al. (1987), with measurement of total C and N in the extracts using a Dimatoc 100 apparatus (Dimatec, Essen, Germany). Microbial C and N ($C_{\text{chi}}$ and $N_{\text{chi}}$) are reported as amounts of C and N rendered extractable by chloroform fumigation, without the use of conversion factors. Soil pH ($n = 2$) was determined after 1 h of equilibration with H$_2$O$_{\text{dest}}$ in a ratio of 1:2.5 (w:v).
Determination of $^{31}$P and $^{33}$P in the microbial biomass

$P$ held in the microbial biomass was determined by simultaneous liquid fumigation and extraction with anion-exchange resin membranes (BDH #55164) in bicarbonate form as described by Kouno et al. (1995), but using hexanol as the fumigant instead of chloroform which was found to dissolve these anion-exchange membranes. Fumigation with hexanol has been shown to be as effective as chloroform fumigation to release microbial $P$ (McLaughlin et al., 1986). Briefly, moist soil equivalent to 2 g dry weight was shaken horizontally with 30 ml $H_2O_{dest}$ and 2 resin membranes ± 1 ml hexanol for 16 h at 170 reciprocations min$^{-1}$. The resin membranes were then rinsed carefully with $H_2O_{dest}$ and eluted with 20 ml 0.5 $M$ HCl. The amount of $^{31}$P extracted from fumigated samples in addition to non-fumigated samples has to be corrected for sorption of released $^{31}$P during the extraction period as determined with additional non-fumigated subsamples receiving a known addition of inorganic $^{31}$P. The recovery of added $^{31}$P is described by a linear function in these soils, at least for additions of up to 50 mg $P$ kg$^{-1}$ soil (data not shown), and a single $^{31}$P spike is therefore sufficient to correct for $P$ sorption.

Microbial $^{31}$P ($^{31}$P$_{hex}$) is then calculated as

$$^{31}$P$_{hex}$ (mg P kg$^{-1}$) = ($^{31}$P$_{fum}$ − $^{31}$P$_{resin}$) / $^{31}$P$_{rec}$

(6)

where $^{31}$P$_{fum}$ and $^{31}$P$_{resin}$ are the amounts of $^{31}$P extracted from fumigated and non-fumigated subsamples, respectively, and $^{31}$P$_{rec}$ is the fraction of the $^{31}$P spike that is recovered. Averaged over all dates, $^{31}$P$_{rec}$ amounted to 0.60 and 0.64 for COM and MCF, respectively ($P = 0.000$), showing no significant effect of the added $P$ sources.

McLaughlin et al. (1988) pointed out that the difference in $^{33}$P recovered in $P_{fum}$ and $P_{resin}$ cannot be corrected for sorption of $^{33}$P using the recovery of a $^{33}$P spike, as the amount of $^{31}$P released from the biomass during fumigation affects the recovery of $^{33}$P due to sorption/desorption and isotopic exchange reactions. In our study, the addition of a $^{31}$P spike to labeled soils increased the recovery of $^{33}$P in $P_{resin}$, but in contrast to the results of Oehl et al. (2001a), several pre-tests did not reveal a strictly linear relationship. The added amount of $^{31}$P was therefore kept close to the amount of $^{31}$P$_{hex}$ (SD± 0.5 mg P kg$^{-1}$) as determined in the preliminary incubation. At the same amount of $^{31}$P added, the recovery of $^{33}$P was then linear over a large range of specific activities.
($^{33}$P/$^{31}$P) in the spike, as was observed by Oehl et al. (2001a). Therefore, only one $^{33}$P spike was required which contained the same amount of $^{31}$P as the $^{31}$P spike.

The recovery of $^{33}$P in $P_{\text{hex}}$ ($^{33}$P$_{\text{hex}}$) is calculated as

$$^{33}P_{\text{hex}}(\%) = \frac{(^{33}P_{\text{fum}} - ^{33}P_{\text{resin+P}})}{^{33}P_{\text{rec}}}$$

where $^{33}P_{\text{fum}}$ and $^{33}P_{\text{resin+P}}$ represent the recovery of $^{33}$P in fumigated and $^{31}$P-spiked (non-fumigated) subsamples, respectively, and $^{33}P_{\text{rec}}$ is the fraction of the $^{33}$P spike that is recovered. Averaged over all dates, $^{33}P_{\text{rec}}$ amounted to 0.54 and 0.60 for COM and MCF, respectively ($P = 0.000$), with $^{33}P_{\text{rec}}$ being significantly lower than $^{31}P_{\text{rec}}$ on 4 out of 7 sampling dates, the reason for which remains unclear. Added P sources had a weak effect on $^{33}P_{\text{rec}}$ ($P = 0.039$) which averaged 0.55, 0.57 and 0.58 for soil IEP, added P, and plant residue amendment, respectively. In total, 15 subsamples of soil (equal to 2 g dry weight) were prepared to determine $^{31}$P and $^{33}$P in $P_{\text{hex}}$, with four replicates each for $P_{\text{fum}}$ and $P_{\text{resin}}$ and the $^{31}$P spike, and three that received a $^{33}$P spike, respectively.

**Sequential P fractionation**

On days 10, 30, and 51, a separate set of samples ($n = 4$) was prepared to determine the fate of $^{33}$P beyond $P_{\text{fum}}$. The first extraction was similar to the treatment of samples for $P_{\text{fum}}$ in the method for $P_{\text{hex}}$, but using half the amount of soil, $H_2O_{\text{dest}}$, hexanol, and one resin membrane in a 50 ml centrifuge tube. After shaking, the resin membrane was rinsed with 10 ml $H_2O_{\text{dest}}$ above the sample. The volume of water in the tube was adjusted to 29 ml by weight and 1 ml 3 M NaOH was added, resulting in 30 ml of 0.1 M NaOH. The next steps were done according to the protocol by Tiessen and Moir (1993): After shaking overnight, the samples were centrifuged for 10 min at 25000 g and the supernatant vacuum-filtered through a millipore filter (Sartorius, cellulose acetate, pore size 0.2 μm). An aliquot of 5 ml was acidified to pH 1.5 by adding 0.6 ml 1 M $H_2SO_4$, kept at 4°C for 30 min and centrifuged to separate the precipitated organic matter from the clear supernatant. Another 2 ml were digested on a hot plate with 0.7 g $K_2S_2O_8$, 1 ml 11 N $H_2SO_4$ and $H_2O_{\text{dest}}$ as required until the solution was clear. The concentration of $P_i$ in the supernatant (NaOH-$P_i$) and in the digested NaOH-extract (NaOH-$P_i$) was measured colorimetrically after neutralization (Murphy and Riley, 1962). Organic P ($P_o$)
extracted with NaOH (NaOH-P<sub>0</sub>) was calculated as the difference between NaOH-P<sub>t</sub> and NaOH-P<sub>i</sub>.

The total radioactivity extracted with NaOH was determined by liquid scintillation as described above, but after dilution of the colored extract with H<sub>2</sub>O<sub>dest</sub> (1:4). A small color quenching effect (4-6%) as revealed by standard additions of 33P to the extract was corrected for. The separation of 32P<sub>i</sub> and 33P<sub>o</sub> in the NaOH-extract for counting using acidified molybdate and isobutanol (Jayachandran et al., 1992) was tested. However, the brown streaks of humic material in the extract were observed to move into both the aqueous and the organic phase, suggesting that the separation of P<sub>i</sub> and P<sub>o</sub> was not complete. Therefore, the difference between 33P in the supernatant (in which NaOH-P<sub>i</sub> was determined) and in the NaOH-extract was assumed to represent 33P-labeled NaOH-P<sub>o</sub>. This separation of 33P<sub>i</sub> and 33P<sub>o</sub> is probably not complete, as P<sub>i</sub> may precipitate along with organic matter during the acidification-centrifugation step, while on the other hand some organic materials (e.g. fulvic acids) remain in the supernatant (Tiessen and Moir, 1993). Therefore, an additional method was tried, using the above-mentioned anion-exchange resin membranes to extract 33P<sub>i</sub> from the NaOH-extract. To this end, 10 resin membranes (saturated with HCO<sub>3</sub> ) were added to 5 ml NaOH-extract diluted with 40 ml H<sub>2</sub>O<sub>dest</sub> and put on an overhead shaker for 24 h. Resin membranes were rinsed briefly with H<sub>2</sub>O<sub>dest</sub> and eluted with 0.5 M HCl. On each date, 83-84% of 31P and 33P from standard additions to selected samples (n = 3, CV 1-3%) were recovered on the resin membranes. The percentage of 33P in the NaOH-extract recovered on resin membranes (NaOH-P<sub>resin</sub>) is presented after correction for the incomplete recovery of standard additions.

**Conditions of the pot experiment**

Due to its importance as the main crop in western Kenya, maize was chosen as the test plant. One week before the beginning of each growth phase, maize seeds (H513, Kenya Seed Company) weighing 0.45-0.50 g were surface-sterilized with 5% Ca(ClO)<sub>2</sub> for 5 min and washed in H<sub>2</sub>O<sub>dest</sub> for 15 min before germinating on quartz sand at 33°C. After 2-3 days, seedlings were placed on folded filter papers with the tip cut off so that the roots could reach down into a box with H<sub>2</sub>O<sub>dest</sub> in the climate chamber. As the seed represents a significant source of P that may be utilized preferentially under conditions
of low P availability (Truong and Pichot, 1976; Bühler et al., 2003), the remainder of the seed was removed from each seedling using a scalpel before transplanting one seedling into each pot at the beginning of each growth phase.

In order to estimate the amount of P in the seedling at transplanting, a correlation between fresh weight of seedlings (after removal of the seed) and P content \( (n = 15) \) was established, and was significant for each growth phase. During the 21 days of maize growth, pots were watered daily to replace water losses by evapotranspiration. In order to check for a potential influence of the plants on the microbial biomass, a soil core was taken from each pot at harvest of the first growth phase and \( P_{\text{hex}} \) was determined on a composite sample for each of the six combinations of P source and soil.

**Plant analysis**

After cutting the maize shoots at harvest, the bottles were filled with water, closed with a lid and shaken horizontally at 180 reciprocations for 15 min. The root system could then be removed easily and washed free from soil by shaking in water twice for 30 min each. The P concentration and radioactivity in shoots and roots were determined by dry combustion as described above for the labeled crotalaria residue. N concentrations were determined on ball-milled subsamples of shoots from four and roots from two plants, respectively, for each of the six combinations of P source and rotation and for each growth phase.

**Statistical analysis**

Statistical analysis was carried out with SYSTAT (SPSS 2000). A t-test was performed to check for differences in soil properties between crop rotations in the field samples. For each measuring date of the incubation experiment, soil respiration and microbial analyses were tested by two-way ANOVA with the factors P source, crop rotation, and the P source x crop rotation interaction. Results from each growth phase during the pot trial were analyzed in the same way. Data from the incubation experiment were also subjected to three-way ANOVA with the factors P source, crop rotation, sampling date, and all possible interactions. Multiple comparisons using Tukey’s test were done whenever the ANOVA indicated significant differences \( (P \leq 0.05) \).
Results

Soil respiration, microbial C and N and soil pH

For all P sources, soil respiration was significantly higher in MCF than in COM (Table 4.4). Addition of P_i did not affect soil respiration, and the additional amount of CO_2 released from residue-amended soils was similar for both rotations, amounting to 55% of added C after 63 days. C\textsubscript{chl} did also not change in response to P_i addition, except for a small but significant decrease on day 21, whereas the addition of the residue increased C\textsubscript{chl} by a factor of 1.3-1.6 throughout the incubation. Likewise, N\textsubscript{chl} was significantly increased by residue addition (not shown). On day 21, soil pH was 0.2 units lower in the treatments with addition of nutrient solution (added P_i and soil IEP) than in the residue-amended soils in which pH was the same as in the original soils. The difference subsequently diminished as pH values in residue-amended soils approached those in the other treatments (not shown).

Table 4.4: Treatment effects on soil respiration and microbial C

<table>
<thead>
<tr>
<th>P source</th>
<th>rotation</th>
<th>—— respiration ——</th>
<th>—— C\textsubscript{chl} ——</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>day 0-63</td>
<td>21</td>
</tr>
<tr>
<td>soil IEP</td>
<td>COM</td>
<td>153 d</td>
<td>82 d</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>286 c</td>
<td>132 b</td>
</tr>
<tr>
<td>added P\textsubscript{i}</td>
<td>COM</td>
<td>162 d (in addition)</td>
<td>76 e</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>289 c (to soil IEP)</td>
<td>114 c</td>
</tr>
<tr>
<td>plant residue</td>
<td>COM</td>
<td>928 b (ns)</td>
<td>130 b</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>1086 a</td>
<td>169 a</td>
</tr>
</tbody>
</table>

source of variation

- **P source (P)**: *** *** *** ***
- **rotation (R)**: *** *** *** ***
- **P x R**: ns ** ns

* P = 0.01-0.05, ** P = 0.01-0.001, *** P < 0.001, ns = not significant

Within columns, means followed by the same letter are not significantly different (P = 0.05) by Tukey’s multiple range test.
Figure 4.2: Amount of P, recovery of $^{33}$P and relative specific activity in $P_{hex}$ (left) and $P_{resin}$ (right); bars show Tukey’s HSD$_{0.05}$
**Microbial and resin-extractable P**

On each sampling date, levels of $P_{\text{hex}}$ were significantly increased above the other two P sources by addition of the plant residue and those of $P_{\text{resin}}$ by addition of $P_i$ (Figure 4.2). At the same amount of P added (6 mg P kg$^{-1}$), the maximum change in $P_{\text{hex}}$ (by residue amendment) and $P_{\text{resin}}$ (by $P_i$ addition) amounted to 2.9 and 1.8 mg P kg$^{-1}$, respectively. $P_{\text{hex}}$ was always higher and $P_{\text{resin}}$ lower in MCF than in COM. For each of the six combinations of P source and soil, $P_{\text{resin}}$ was higher on day 1 than on most other dates, with the decrease being most pronounced after addition of $P_i$. In the case of $P_{\text{hex}}$, no time trend was observed.

Table 4.5: Main treatment effects on $P_{\text{hex}}$ and $P_{\text{resin}}$ (amount of P, recovery of $^{33}$P, and P derived from the labeled sources) and significance of treatment interactions

<table>
<thead>
<tr>
<th>factor</th>
<th>— amount —</th>
<th>— recovery of $^{33}$P —</th>
<th>— P derived from source —</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_{\text{hex}}$</td>
<td>$P_{\text{resin}}$</td>
<td>$P_{\text{hex}}$</td>
</tr>
<tr>
<td><strong>P source (P)</strong></td>
<td>mg kg$^{-1}$</td>
<td>% of added</td>
<td>%</td>
</tr>
<tr>
<td>soil IEP</td>
<td>3.9 c</td>
<td>1.0 b</td>
<td>6.7 b</td>
</tr>
<tr>
<td>added $P_i$</td>
<td>4.1 b</td>
<td>1.7 a</td>
<td>3.6 c</td>
</tr>
<tr>
<td>plant res.</td>
<td>5.9 a</td>
<td>1.0 b</td>
<td>15.0 a</td>
</tr>
<tr>
<td><strong>rotation (R)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COM</td>
<td>3.3 b</td>
<td>1.4 a</td>
<td>7.2 b</td>
</tr>
<tr>
<td>MCF</td>
<td>6.0 a</td>
<td>1.1 b</td>
<td>9.6 a</td>
</tr>
<tr>
<td><strong>day (D)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.9 ab</td>
<td>1.8 a</td>
<td>10.2 a</td>
</tr>
<tr>
<td>10</td>
<td>5.0 a</td>
<td>1.2 b</td>
<td>8.2 ab</td>
</tr>
<tr>
<td>21</td>
<td>4.7 b</td>
<td>1.1 c</td>
<td>8.9 ab</td>
</tr>
<tr>
<td>30</td>
<td>4.4 c</td>
<td>1.1 bc</td>
<td>7.9 b</td>
</tr>
<tr>
<td>42</td>
<td>4.4 c</td>
<td>1.1 bc</td>
<td>7.9 b</td>
</tr>
<tr>
<td>51</td>
<td>4.8 ab</td>
<td>1.1 bc</td>
<td>8.1 ab</td>
</tr>
<tr>
<td>63</td>
<td>4.2 c</td>
<td>1.1 bc</td>
<td>8.0 b</td>
</tr>
</tbody>
</table>

**significance of interactions:**

<table>
<thead>
<tr>
<th>interaction</th>
<th>ns</th>
<th>ns</th>
<th>***</th>
<th>**</th>
<th>**</th>
<th>ns</th>
<th>ns</th>
<th>ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>P x R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P x D</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>**</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>R x D</td>
<td>**</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>P x R x D</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

n.a. not applicable, * $P = 0.01-0.05$, ** $P = 0.01-0.001$, *** $P < 0.001$, ns = not significant

Within columns and factors, means followed by the same letter are not significantly different ($P = 0.05$) by Tukey’s multiple range test
Figure 4.3: Source of microbial P after plant residue amendment (bars show SD of $P_{\text{hex}}$ in non-amended soils (soil IEP), of soil-derived and total $P_{\text{hex}}$, respectively)

Up to 18% of applied $^{33}P$ was recovered in $P_{\text{hex}}$, with the recovery generally ranging in the order plant residue > soil IEP ≥ added $P_i$, being higher in MCF than in COM except on day 1 (Figure 4.2). A maximum of 22% of $^{33}P$ was recovered in $P_{\text{resin}}$. On each date, the recovery of $^{33}P$ in $P_{\text{resin}}$ followed the order added $P_i$ > soil IEP > residue, and from day 21 onwards, it was higher in COM than in MCF. The percentage of $^{33}P$ found in $P_{\text{hex}}$ did not change significantly in the course of the experiment, while for $P_{\text{resin}}$, a steady decrease was observed in all cases.

Figure 4.2 also shows the resulting relative specific activities which were generally higher in $P_{\text{hex}}$ and lower in $P_{\text{resin}}$ after residue amendment than for the other two P sources. Consequently, a greater percentage and amount of $P_{\text{hex}}$ was derived from the plant residue than from added $P_i$, while the opposite applied for $P_{\text{resin}}$ (Table 4.5). The small increase in $P_{\text{hex}}$ after addition of $P_i$ that was significant in the overall analysis of the data was similar to the amount of $P_{\text{hex}}$ derived from this source (0.2 mg P kg$^{-1}$). In the case of plant residue addition, however, additional uptake of soil P was implied. The increase in $P_{\text{hex}}$ above the non-residue-amended soils was greatest on day 10, amounting to 2.8 mg P kg$^{-1}$ irrespective of the rotation (Figure 4.3). At this date, uptake of soil P contributed 1.9 mg P kg$^{-1}$ to the increase in $P_{\text{hex}}$ after residue amendment in both soils, decreasing to 0.8 and 0.6 mg P kg$^{-1}$ at the end of the incubation in COM and MCF,
respectively. For both rotations, the amount of $P_{hex}$ derived from the residue remained at 0.9-1.0 mg P kg$^{-1}$ throughout the incubation.

**Sequential P fractionation**

In the separate set of samples for sequential P fractionation, similar amounts of P and percentages of $^{33}$P were extracted in $P_{fum}$ as during the determination of $P_{hex}$. In the succeeding extraction with NaOH, the distribution of total amounts of P into $P_i$ and $P_o$ differed between rotations, with a shift towards $P_o$ observed in MCF (Table 4.6). Neither NaOH-$P_i$ nor the sum of $P_{fum}$ and NaOH-$P_i$ were affected by rotations or amendments. Slightly lower amounts of NaOH-$P_i$ and total P were extracted on day 10 than on the other two dates. The recovery of $^{33}$P in NaOH-$P_i$ was similar on all dates, being significantly lower after addition of $P_i$ (66%) than after labeling of soil IEP (71%)

**Table 4.6: Main treatment effects on the amount of P and percentage of $^{33}$P extracted during the sequential fractionation and significance of treatment interactions**

<table>
<thead>
<tr>
<th>factor</th>
<th>amount of P</th>
<th>recovery of $^{33}$P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_{fum}$</td>
<td>NaOH sum</td>
</tr>
<tr>
<td></td>
<td>mg P kg$^{-1}$</td>
<td>% of applied</td>
</tr>
<tr>
<td>$P$ source ($P$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>soil IEP</td>
<td>3.7 c</td>
<td>71 b</td>
</tr>
<tr>
<td>added $P_i$</td>
<td>4.6 b</td>
<td>74 a</td>
</tr>
<tr>
<td>plant residue</td>
<td>5.1 a</td>
<td>69 b</td>
</tr>
<tr>
<td>rotation ($R$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COM</td>
<td>3.6 b</td>
<td>76 a</td>
</tr>
<tr>
<td>MCF</td>
<td>5.4 a</td>
<td>67 b</td>
</tr>
<tr>
<td>day ($D$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.5 ns</td>
<td>69 b</td>
</tr>
<tr>
<td>30</td>
<td>4.5 ns</td>
<td>72 a</td>
</tr>
<tr>
<td>51</td>
<td>4.4 ns</td>
<td>73 a</td>
</tr>
</tbody>
</table>

**Significance of interactions:**

- $P \times R$: ns ns ns ns ns ns ns ns
- $P \times D$: ** ns ns ns ns ns ns ns
- $R \times D$: ns * ns ns ns ns ns
- $P \times R \times D$: ns * ns ns ns ns ns

* $P = 0.01$-$0.05$, ** $P = 0.01$-$0.001$, ns = not significant
Within columns and factors, means followed by the same letter are not significantly different ($P = 0.05$) by Tukey’s multiple range test.
Chapter 4: Microbial and plant uptake of P from different sources

Figure 4.4: Recovery of $^{33}\text{P}$ in the sequential extraction scheme ($\text{P}_{\text{resin}}$ determined separately; bars show SD of the sum of $^{33}\text{P}$ recovered in $\text{P}_{\text{film}}$ and NaOH-P$_1$)

or residue amendment (76%). In total, 76-90% of the applied radioactivity was recovered in the two sequential extractions, with the highest recovery after residue addition and no effect of rotation or time of incubation. Figure 4.4 summarizes the distribution of $^{33}\text{P}$ over the extracted pools at the three sampling dates, additionally showing the recovery in $\text{P}_{\text{resin}}$ as determined during the measurement of P$_{\text{hex}}$.

The two attempts to separate $^{33}\text{P}_{\text{i}}$ and $^{33}\text{P}_{\text{o}}$ in the NaOH-extract indicated significant interactions between P source and rotation (Table 4.7). In the case of residue amendment, the percentage of $^{33}\text{P}$ recovered in NaOH-P$_1$ or NaOH-P$_{\text{resin}}$ did not differ between soils, whereas with P$_i$ addition and labeling of soil IEP, the recovery in the inorganic fraction was 2-5% lower in MCF than in COM. With both methods, the recovery of $^{33}\text{P}_{\text{i}}$ from the NaOH-extract ranged in the order added P$_i$ $\geq$ soil IEP $\geq$ residue and decreased during the incubation.

For labeling of soil IEP, the recovery of $^{33}\text{P}$ from the NaOH-extract in NaOH-P$_i$ and NaOH-P$_{\text{resin}}$ was similar. After addition of P$_i$, 2-3% more were recovered in NaOH-P$_{\text{resin}}$ than in NaOH-P$_i$. The greatest difference between the two methods was observed in the case of plant residue addition, where 10% less of $^{33}\text{P}$ in the NaOH-extract was recovered in NaOH-P$_{\text{resin}}$ than in NaOH-P$_i$. 
Table 4.7: Percentage of $^{33}$P in the NaOH-extract recovered in NaOH-P$_i$ and on anion-exchange resin membranes

<table>
<thead>
<tr>
<th>factor</th>
<th>$%$ of $^{33}$P in NaOH-P$_i$</th>
<th>t-test$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaOH-P$_i$</td>
<td>NaOH-P$_{resin}$</td>
</tr>
<tr>
<td>$P$ source$^b$ (P)</td>
<td>rotation$^b$ (R)</td>
<td></td>
</tr>
<tr>
<td>soil IEP</td>
<td>COM</td>
<td>96 b</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>92 c</td>
</tr>
<tr>
<td>added $P_i$</td>
<td>COM</td>
<td>98 a</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>96 b</td>
</tr>
<tr>
<td>plant residue</td>
<td>COM</td>
<td>83 d</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>84 d</td>
</tr>
<tr>
<td>day (D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>93 a</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>92 b</td>
</tr>
<tr>
<td>51</td>
<td></td>
<td>90 c</td>
</tr>
</tbody>
</table>

significance of other interactions:

<table>
<thead>
<tr>
<th></th>
<th>P x D</th>
<th>R x D</th>
<th>P x R x D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>*</td>
</tr>
</tbody>
</table>

* $P = 0.01$-$0.05$, ** $P = 0.01$-$0.001$, *** $P < 0.001$, ns = not significant
Within columns and factors, means followed by the same letter are not significantly different ($P = 0.05$) by Tukey’s multiple range test

$^a$ t-test between NaOH-P$_i$ and NaOH-P$_{resin}$

$^b$ The interaction between P source and rotation was highly significant in all cases

**Pot experiment**

During the first growth phase, total plant dry matter was significantly affected by the P sources, following the treatment order added $P_i >$ soil IEP > residue (Table 4.8). A negative interaction between rotation MCF and residue amendment occurred which was also observed during the second growth phase. During the third growth phase, plant dry matter was similar in all treatments. Between 0.1-2% of applied $^{33}$P was recovered in the plants, with a significantly higher recovery from added $P_i$ than from the other two P sources during each growth phase. Rotations generally did not affect the recovery of $^{33}$P, except for significant interactions with addition of $P_i$ during the first growth phase and with residue addition during the second. The only significant effect on plant P and N content was an increase by addition of $P_i$ during the first growth phase.
Table 4.8: Treatment effects on dry matter, recovery of $^{33}$P, plant P and N content in the pot trial

<table>
<thead>
<tr>
<th>P source</th>
<th>growth phase</th>
<th>dry matter</th>
<th>recovery of $^{33}$P</th>
<th>P content</th>
<th>N content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rotation</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
<tr>
<td>soil IEP</td>
<td>COM</td>
<td>0.92 ab 0.73 a 0.48 ns</td>
<td>0.27 c 0.13 c 0.11 b</td>
<td>0.58 b 0.47 ns 0.33 ns</td>
<td>18.9 ab 14.5 ns 8.9 ns</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>0.91 b 0.73 a 0.48 ns</td>
<td>0.21 c 0.10 c 0.10 b</td>
<td>0.62 ab 0.49 ns 0.36 ns</td>
<td>19.0 ab 14.6 ns 10.4 ns</td>
</tr>
<tr>
<td>added P 1</td>
<td>COM</td>
<td>1.12 a 0.83 a 0.52 ns</td>
<td>1.46 b 0.70 a 0.41 a</td>
<td>0.69 a 0.53 ns 0.41 ns</td>
<td>21.6 a 14.4 ns 9.2 ns</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>1.08 ab 0.71 a 0.45 ns</td>
<td>2.01 a 0.78 a 0.37 a</td>
<td>0.69 a 0.56 ns 0.36 ns</td>
<td>21.6 a 14.9 ns 9.2 ns</td>
</tr>
<tr>
<td>plant res.</td>
<td>COM</td>
<td>0.92 ab 0.74 a 0.51 ns</td>
<td>0.56 c 0.50 b 0.25 ab</td>
<td>0.55 b 0.55 ns 0.42 ns</td>
<td>15.8 b 14.3 ns 9.1 ns</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>0.63 c 0.42 b 0.32 ns</td>
<td>0.43 c 0.19 c 0.15 b</td>
<td>0.56 b 0.49 ns 0.33 ns</td>
<td>16.6 b 12.0 ns 8.8 ns</td>
</tr>
</tbody>
</table>

*source of variation*

- **P source (P)**
  - *** P < 0.001
  - ** P < 0.01
  - * P = 0.01-0.05
  - ns not significant

- **Rotation (R)**
  - ** P < 0.01
  - * P = 0.01-0.05
  - ns not significant

- **P x R**
  - * P = 0.01-0.05
  - ns not significant

*Within columns, means followed by the same letter are not significantly different (P = 0.05) by Tukey’s multiple range test.*
Plant dry matter, nutrient contents and the recovery of $^{33}$P in the plant decreased steadily with succeeding growth phases (Table 4.8). The estimated P content of transplanted seedlings also decreased from 0.77 (SD = 0.11) during the first and 0.65 (SD = 0.12) during the second to 0.40 (SD = 0.11) mg P plant$^{-1}$ during the third growth phase. The reason for the decrease in fresh weight of seedlings and resulting estimated P content is not known, but germination was observed to become slower. The estimated P content at transplanting was higher than the amount of P determined in the plants at harvest, except for the third growth phase where the difference was not significant. However, equally treated maize seedlings ($n = 6$) that were planted on a Swiss grassland soil high in P$_{resin}$ (88 mg P kg$^{-1}$ soil) took up 5.0 (SD = 1.1) mg P plant$^{-1}$ in addition to the estimated amount of P in transplanted seedlings during the third growth phase. Therefore, the absence of net P uptake from the Kenyan soils cannot be attributed to the stress imposed by seed removal. In addition, plant P uptake is indicated by the low but significant recovery of $^{33}$P in the plants.

A greater percentage of plant P was derived from added P$_i$ (2-6%) than from the residue (1-2%), showing no consistent rotation effect (Table 4.9). This would translate into a maximum amount of P derived from a labeled P addition of 0.04 mg plant$^{-1}$ in the case of P$_i$ added to MCF during the first growth phase. Obviously, this is too little to be measurable against the average estimated P content of transplanted seedlings of 0.77 mg. The decrease in seedling vigor may have contributed to the decrease in $^{33}$P uptake in the course of the experiment (Table 4.8). Temporal trends of $^{33}$P in the plant can therefore not be evaluated, except for the observation that the decrease in $^{33}$P recovered from added P$_i$ between growth phase 1 and 3 tended to be more pronounced than for the other two P sources.

**Microbial and resin-extractable P in the presence of the plant**

At the end of the first growth phase, levels of P$_{hex}$ and P$_{resin}$ and the recovery of $^{33}$P in P$_{hex}$ were similar in incubated soils and samples taken from the planted pots (not shown). The only significant effect of the presence of plants was a slight decrease in $^{33}$P recovered in P$_{resin}$ ($P = 0.036$). Analyses done on incubated soils therefore appear to represent a reasonable baseline for the pot trial.
Table 4.9: Percentage of plant P derived from labeled sources

<table>
<thead>
<tr>
<th>P source</th>
<th>growth phase</th>
<th>rotation</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>added $P_i$</td>
<td>COM</td>
<td></td>
<td>4.1 b</td>
<td>2.7 a</td>
<td>1.9 ab</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td></td>
<td>5.6 a</td>
<td>2.7 a</td>
<td>2.0 a</td>
</tr>
<tr>
<td>plant residue</td>
<td>COM</td>
<td></td>
<td>1.9 c</td>
<td>1.8 b</td>
<td>1.2 bc</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td></td>
<td>1.5 c</td>
<td>0.7 c</td>
<td>0.8 c</td>
</tr>
</tbody>
</table>

**source of variation**

|  | P source ($P$) | Rotation (R) | P x R |
|  | ***           | *            | ns    |

* $P = 0.01-0.05$, ** $P = 0.01-0.001$, *** $P < 0.001$, ns = not significant

Means followed by the same small letter (within columns) or by the same capital letter (within rows) are not significantly different ($P = 0.05$) by Tukey’s multiple range test

Discussion

Recovery of $P$ sources in the microbial biomass

The addition of a plant residue caused $P_{hex}$ to increase by a factor of 1.5 and resulted in the greatest recovery of $^{33}P$, averaging 15% compared to 7% after carrier-free labeling of soil IEP and 4% after addition of $P_i$ (Table 4.5). In the pot experiment of McLaughlin and Alston (1986), the recovery of label in the microbial biomass was also greater from a plant residue than from added $P_i$, amounting to 68 and 23%, respectively. As they used a conversion factor of 0.4, this translates into 27 and 9% of isotope rendered extractable by fumigation. Oehl et al. (2001a) observed that two days after carrier-free labeling of soil, 66% of the tracer was recovered in chloroform-extractable P when soils were amended with glucose and ammonium nitrate, compared to 8% in the absence of easily available sources of C and N. Thus, the results from these studies agree with our observation that the stimulation with a C substrate boosts the recovery of label in the microbial biomass.

Although compared to labeling of soil IEP, the small amount of $P_i$ added (6 mg P kg$^{-1}$) generally did not affect the amount of $P_{hex}$ (Figure 4.2) or microbial activity as indicated by soil respiration (Table 4.4), the recovery of $^{33}P$ in $P_{hex}$ was lower from added $P_i$ (4 vs. 7%, $P = 0.000$). Relatively little is known about the forms of $P$ taken up by
microorganisms in soil, but it is generally assumed that orthophosphate in the soil solution represents the most important source of P for microbial uptake. We could not determine the specific activity of $P_i$ in the soil solution because in the strongly P sorbing soil used in our study, the concentration of $P_i$ in the soil solution is close to the detection limit, and less than 2% of the added isotope is recovered in the soil solution after one minute of isotopic exchange (chapter 1). Instead, $P_{resin}$ was measured, a pool which has been reported to contain $P_i$ that is isotopically exchangeable within a time frame equivalent to the duration of the resin extraction, at least in a P-rich loamy soil (Schneider and Morel, 2000). One day after soil amendment, 22% of $^{33}P$ added with $P_i$ was recovered in $P_{resin}$, compared to 12% after carrier-free labeling of soil P (Figure 4.2). The addition of $P_i$ also increased the amount of $P_{resin}$ by 1.8 mg P kg$^{-1}$, and the resulting specific activity of $P_{resin}$ was lower than after labeling of soil IEP (0.07 vs. 0.10, $P = 0.002$). If the specific activity of $P_{resin}$ is similar (or directly related) to the specific activity of the source of microbial P uptake, this difference may explain the lower recovery of $^{33}P$ in $P_{hex}$ after addition of $P_i$. However, from day 10 onwards, the specific activity in $P_{resin}$ did not differ between the two treatments.

At the same amount of P added, the level of $P_{resin}$ as well as the recovery of $^{33}P$ and specific activity in $P_{resin}$ were lower after plant residue than after $P_i$ addition (Figure 4.2). When the plant residue was extracted with resin-membranes in the absence of soil, 69% of $^{31}P$ and 87% of $^{33}P$ were recovered in $P_{resin}$, reflecting the higher specific activity in $P_{resin}$ than in $P_{tot}$ (Table 4.2). Although we do not know if hydrolysis of $P_o$ occurred during the extraction, which may proceed less rapidly when the dry residue is incorporated into the soil, the plant residue can be considered as a mixture of $P_i$, $C$, and some $P_o$. For the comparison of P added as $P_i$ or with a plant residue, it is then mainly the presence of C that affects the recovery of $^{33}P$ not only in $P_{hex}$, but also in $P_{resin}$.

Compared to carrier-free labeling of soil IEP, the recovery of $^{33}P$ in $P_{resin}$ was lower after plant residue addition, while levels of $P_{resin}$ were generally similar. Nevertheless, considerable microbial uptake of soil P is suggested by the calculated amount of $P_{hex}$ derived from the plant residue, which represented less than half of the absolute increase in $P_{hex}$ after 10 days (Figure 4.3). For this calculation, the specific activity of the whole residue was used. Assuming that only the resin-extractable fraction of the plant residue would have been available to microorganisms during this period, the higher specific
activity in this fraction would result in even lower amounts of residue-derived $P_{\text{hex}}$. The results of McLaughlin and Alston (1986) also suggest a significant uptake of soil P into the microbial biomass 34 days after amendment with a plant residue of low quality (C:P-ratio $\sim$400). In our study, the C:P-ratio of the residue (240) was above the often cited threshold of 200 (Dalal, 1977), and soil P immobilization could therefore be expected. In a previous study with the same soil, addition of a crotalaria residue of higher quality (C:P-ratio 176) did decrease levels of $P_{\text{resin}}$ during the first few weeks after addition (chapter 2). Most probably, an apparent depletion of $P_{\text{resin}}$ was absent in the current study because the amount of plant residue added was smaller (3.3 g DM kg$^{-1}$ compared to 5.9 in the previous study), reducing the extent of microbial depletion of $P_{\text{resin}}$ which could then be buffered by replenishment from other soil P pools. This would also explain the lower specific activity in $P_{\text{resin}}$ after residue amendment, because throughout the incubation, the specific activity in NaOH-$P_i$ was lower than that in $P_{\text{resin}}$. This agrees with the observation by Bühler et al. (2002) of decreasing specific activities in $P_i$ fractions in the order of extraction. In our study, up to 90% of the total applied radioactivity was recovered during the two sequential extractions, while at the same time only 40% of total P in these soils (720 mg kg$^{-1}$) was extracted, suggesting that the specific activity in the non-extracted P would be very low.

**Separation of $^{33}P$ labeled $P_i$ and $P_o$**

Both methods used to separate $^{33}P$ labeled $P_i$ and $P_o$ in the NaOH extract gave the same result that for non-residue amended soils, the proportion of label in the NaOH extract recovered in the inorganic fraction was lower in MCF than in COM (Table 4.7). In the absence of plant residue addition, $^{33}P_o$ can only be of microbial origin. The recovery of microbial P during the previous fumigation-extraction step is probably incomplete not only due to P sorption, but also due to incomplete hydrolysis of microbial $P_o$ as well as incomplete lysis and extraction of microbial cells. The greater proportion of $^{33}P_o$ in MCF is then in agreement with the higher microbial activity in MCF. Also the trend for a higher proportion of $^{33}P$ in the organic fraction after labeling of soil IEP than after addition of $P_i$ corresponds with the recovery of $^{33}P$ from the two sources in $P_{\text{hexo}}$, which is only partly recovered in $P_{\text{fum}}$. 
The use of anion-exchange resin membranes for the separation of $^{32}$P$_i$ and $^{33}$P$_o$ in the NaOH extract is not satisfying due to the incomplete recovery of standard additions of P$_i$ to the extract. In addition, resin membranes have also been used to extract organic anions from soil (Szmigielska et al., 1996; George et al., 2002), and sorption of P$_o$ on the membranes and subsequent elution cannot be excluded. Nevertheless, treatment effects on the recovery of $^{33}$P in NaOH-P$_i$ and NaOH-P$_{resin}$ were generally similar (Table 4.7), except for the residue-amended soils, where a lower proportion of $^{33}$P was recovered in NaOH-P$_{resin}$ than in NaOH-P$_i$. Possibly, some $^{33}$P$_o$ remained in the supernatant during the acidification and centrifugation of the NaOH-extract used to yield a clear supernatant for colorimetric determination of NaOH-P$_n$, as suggested by Tiessen and Moir (1993).

**Recovery of $^{33}$P in the plant**

During the last week of each growth phase, the color of maize leaves turned dark green to purple, and the final concentration of P in shoots and roots was generally below 1 g kg$^{-1}$, indicating P deficiency. The significant effect of P$_i$ addition on plant dry matter and nutrient content observed during the first growth phase is also in agreement with the good response of maize to P fertilization observed in the field (chapter 1) as well as with the increase in P$_{resin}$ (Table 4.5). Until the end of the experiment, P$_{resin}$ remained higher after addition of P$_i$ than in the other two treatments (Figure 4.2), but obviously, the difference ($< 1$ mg P kg$^{-1}$) was too small to affect plant dry matter and nutrient content during the second and third growth phase (Table 4.8). The recovery of $^{33}$P applied with P$_i$, however, was always significantly higher than that from the other P sources. This is in accordance with the strong influence of carrier application on specific activities in the soil solution or other pools of labile P$_i$, especially in highly P sorbing soils (Wolf et al., 1986). In a pot trial with an Oxisol from Colombia, 74% of the P in maize shoots after 11 weeks was derived from an application of $^{33}$P with about 10 mg P kg$^{-1}$ as KH$_2$PO$_4$, while very little uptake of soil P was suggested, as a part of plant P originated from the seed (Bühler, 2001). The non-significant trend for plant uptake of $^{33}$P from the added residue to be equal or even higher than that from soil IEP is more surprising, as the specific activities in P$_{resin}$ were lower after residue addition than in the treatment soil IEP. This would suggest that plants obtained P from other sources than P$_{resin}$. 

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*Chapter 4: Microbial and plant uptake of P from different sources*
While the absence of net P uptake limits the interpretation of our experiment, it is in accordance with another study using an unfertilized, highly P sorbing Oxisol, where total P uptake (sum of three cuts) in shoots of common bentgrass (*Agrostis capillaris*) grown for 12 weeks amounted to only a quarter of the P applied with the seeds (Bühler et al., 2003). P acquisition by maize in an Oxisol from Brazil appeared to depend entirely on mycorrhizal association (Cardoso, 2002). While we did not exclude mycorrhiza, the small volume of soil and the short growth periods may have limited potential benefits of mycorrhizal colonization.

The main advantage of our approach is the elimination of seed P as an unknown fraction of total P in the plant. The apparent loss of P in harvested plants compared to transplanted seedlings may be due to incomplete recovery of the root system or through loss of P from damaged roots during the washing process. Alternatively, a transfer of P from the roots to the soil may have occurred with root exudates or root turnover during plant growth. In wheat, the release of phosphate from the root apex is suggested as a mechanism to enhance resistance to Al toxicity (Pellet et al., 1996).

Maize growing in the Swiss grassland soil produced four times more dry matter than plants on the carrier-free labeled Kenyan soil, while N and P content were increased by a factor of 2.3 and 15.5, respectively, again suggesting P as the primary limiting nutrient in our experiment. The apparent negative interaction between plant residue amendment and soil MCF on dry matter production was presumably caused by an inherent difference between soils COM and MCF that was eliminated by the addition of the nutrient solution in non-residue amended treatments. This difference cannot be attributed to P or N availability, because a) the leaf color of plants on the residue-amended soil MCF was light green rather than purple, b) P and N contents as well as the recovery of $^{32}$P (except for the second growth phase) were similar from residue-amended COM and MCF, c) for the residue treatment, levels of total N extractable with K$_2$SO$_4$ from non-fumigated samples in MCF surpassed those in COM by 6 mg N kg$^{-1}$ throughout the study, and d) a few left-over seedlings ($n = 4$) grown on the original soils COM and MCF without addition of nutrient solution during the second growth phase showed the same differences in leaf color and dry matter production, while P and N content were similar. Thus, the availability of another macro- or microelement appears to have limited plant growth more on MCF than on COM.
In the remaining sections, the experimental factor ‘rotation’ and the temporal dynamics are discussed only with respect to soil P pools, because the problems of the pot trial discussed above do not allow any further interpretation.

**Comparison of soils from the two crop rotations**

In contrast to a previous study (chapter 2), neither the amount of CO₂ released from residue-amended in addition to non-amended soils nor the net change in microbial P differed between COM and MCF. Apparently, the microbial biomass in both soils was able to respond similarly to the lower amounts of plant residue added. For all P sources, however, the soils differed in the recovery of $^{33}$P in $P_{hex}$. Oberson et al. (2001) also observed different percentages of label uptake by the microbial biomass in carrier-free labeled soils from different land-use systems. In their study, the resulting specific activities differed by a factor of 2-3 for two soils with similar levels of microbial biomass, while in our case, the specific activities in $P_{hex}$ were similar for MCF and COM, indicating that the recovery of $^{33}$P in $P_{hex}$ was proportional to the pool size.

**Temporal dynamics in the recovery of $^{33}$P in soil pools**

In accordance with the principles of isotopic exchange, the recovery of $^{33}$P in $P_{resin}$ diminished steadily, decreasing from 22, 12 and 7% on day 1 to 4, 3, and 2% at the end of the incubation for added P, soil IEP and plant residue, respectively, with the greatest decrease occurring between day 1 and 10 (Figure 4.2). The recovery of label in $P_{resin}$ also decreased during incubation of $^{33}$P labeled Oxisols from Colombia (Bühler et al., 2002) and of temperate soils amended with $^{33}$P labeled soybean residues (Daroub et al., 2000). In these studies, a simultaneous increase in the recovery in other sequentially extracted pools such as P extractable with 0.5 M NaHCO₃ and 0.1 M NaOH was observed, while in our study, the recovery of $^{33}$P in NaOH-P₁ remained unchanged at 70-72% (Table 4.6). This is mainly due to the fact that our first sequential extraction took place after 10 days, compared to extractions performed on the day of labeling in the two studies mentioned above. In addition, NaOH-P₁ in our study was not extracted after $P_{resin}$ but after $P_{fum}$, in which the recovery of $^{33}$P decreased only from 12 to 9% between day 10 and 51. This would not have been detectable against the variation in the recovery of $^{33}$P in NaOH-P₁ (Figure 4.4). The predominance of fast exchange reactions
was also observed in the non-fertilized soil studied by Bühler et al. (2002), whereas in P-fertilized soils of the same mineralogy, isotopic exchange between fractions proceeded more slowly.

Throughout the study, the recovery of $^{33}$P in $P_{\text{hex}}$ did not change significantly for any of the P sources. In contrast, Oehl et al. (2001a) observed an increase in $^{33}$P recovered in microbial P during at least the first three weeks after carrier-free soil labeling, except in the case of a non-fertilized soil in which the recovery remained constant after the first 5 days. It thus appears that under conditions of low P availability, the microbial compartment keeps the isotope against the steady decrease of $^{33}$P in available $P_{i}$ caused by isotopic exchange. Alternatively, the difference between the specific activity in microbial and available P may be too small to detect fluxes.

In the absence of substrate amendment, an activation of the microbial biomass with small amounts of substrates becoming available during stirring and mixing of soils at the onset of the experiment may have passed rapidly and the microorganisms returned to a resting state (de Nobili et al., 2001). This is also indicated by the daily respiration rates, which were 2-3 times higher during the first 24 than during the following 48 hours. Alternatively, efficient internal cycling of $^{33}$P within the microbial population may be indicated, with microbial turnover occurring at a constant specific activity of the biomass. In both cases, the apparent increase in $^{33}$P-labeled NaOH-$P_{o}$ during incubation, especially in the non-residue amended treatments (Figure 4.4), is difficult to explain. It could either be derived from components of cells living at the onset of fumigation that were not recovered in $P_{\text{fum}}$, or from components of dead cells already stabilized on soil surfaces. In any case, the sequential extraction after fumigation represents a conservative estimate of label recovery in $P_{o}$, as phosphatases released from lysed cells may mineralize labile $P_{o}$ during the extraction of $P_{\text{fum}}$. In the absence of previous fumigation in the study of Bühler et al. (2002), a considerable proportion of the label recovered in $P_{o}$ fractions after two weeks (up to 20%) may have been extracted from the living microbial biomass.

In the case of plant residue addition, the amount of $P_{\text{hex}}$ showed a weak trend to decrease from the maximum observed after 10 days, while the recovery of $^{33}$P in $P_{\text{hex}}$ did not change significantly (Figure 4.2). In the field experiment of McLaughlin et al. (1988), the percentage of label from the applied plant residue recovered in microbial P
also remained stable during 95 days, while Kouno et al. (2002) observed a decrease in labeled microbial P between 10 and 60 days after addition of labeled ryegrass. In our study, the additional uptake of P into the biomass between day 1 and 10 appeared to be derived from soil (Figure 4.3). Also during the subsequent decline of \( P_{\text{hex}} \), the amount of P derived from the plant residue remained stable. Even in the case of residue amendment, maximum respiration rates occurred during the first 24 h when most \( ^{33}\text{P} \) was taken up. Thereafter, the specific activities in \( P_{\text{hex}} \) and \( P_{\text{resin}} \) were probably too similar to detect additional uptake of \( ^{33}\text{P} \) into the microbial biomass. Alternatively, the apparent uptake of unlabeled P between day 1 and 10 as well as its subsequent release may reflect differences in the composition of the microbial community, where different organism groups that take up P from different sources dominate during different growth phases.

**Conclusions**

The addition of a plant residue and \( P_i \) increased the amount of microbial and resin-extractable P, respectively. Net changes in the size affected the recovery of \( ^{33}\text{P} \) in these two pools accordingly. The increase in \( P_{\text{hex}} \) after plant residue addition also involved a significant uptake of unlabeled P from soil, although the amount of \( P_{\text{resin}} \) was not decreased. In maize, significantly more \( ^{33}\text{P} \) was recovered from added \( P_i \) than from the plant residue and soil IEP, but the interpretation of the pot trial was limited by a maximum recovery of 2% of applied \( ^{33}\text{P} \) and the absence of net P uptake.

The uptake of \( ^{33}\text{P} \) from all P sources into the microbial biomass appeared to occur mainly within the first 24 h after soil amendment and remained almost unchanged thereafter, whereas in \( P_{\text{resin}} \), the recovery of \( ^{33}\text{P} \) declined steadily. In non-residue amended soils, the proportion of \( ^{33}\text{P}_o \) in the NaOH-extract appeared to increase in the course of the experiment and may either be derived from components of living cells which were not recovered during the previous fumigation-extraction step, or from \( P_o \) accumulating during microbial turnover.
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General discussion and conclusions
The main objective of this thesis was to assess the role of the soil microbial biomass in the dynamics of P in highly weathered soils. A field experiment with different maize-fallow rotations and levels of P fertilization on a Ferralsol in western Kenya was chosen as a model case. It was assumed that the incorporation of fallow biomass would enhance the biological processes of soil P dynamics, while mineral P fertilization would increase the availability of P, and thus the overall productivity of the system, with a positive feedback on the biological processes. In addition to agronomic data, changes in soil properties over a period of 5 years were determined (chapter 1). The soils from the field experiment were then used in several incubation experiments under controlled conditions. It was investigated to what extent microbial nutrient immobilization is affected by the addition of fresh substrates, and if P (and N) cycling is influenced by the initial properties of the microbial biomass as determined by crop rotation and P fertilization (chapter 2). The next step was to compare the potential of non-amended soils from the field experiment to deliver P to the soil solution through biological processes, using growing plants as indicators (chapter 3). Finally, the participation of the microbial biomass in the cycling of added P, and plant residues as well as soil P was investigated with the use of radioisotopes (chapter 4). Also in this experiment, it was attempted to include the plant as the ‘customer’ of research in plant nutrition.

The main findings are discussed and synthesized below. Processes of soil P dynamics as described in the general introduction (Figure 5.1) are compared with a schematic presentation of P dynamics in a maize-legume fallow rotation on a Ferralsol in western Kenya (Figure 5.2), showing amounts of P in plant and soil compartments as determined in the field experiment, and microbial processes as concluded from the incubation experiments.

**Agronomic perspective**

The legume species that was tested in the field experiment (*Crotalaria grahamiana*, subsequently referred to as crotalaria) grew well during the first fallow season, producing significantly more biomass than the natural weedy fallow (Table 1.2). Consequently, greater amounts of nutrients were returned to the soil when the biomass of the crotalaria fallow (including weeds) was incorporated, amounting to 9-10 kg P ha\(^{-1}\) (Figure 5.2). In the subsequent season, maize growing on plots of the maize-crotalaria
fallow rotation (MCF) produced twice as much as maize planted after maize (in the continuous maize system, COM), while the production of maize after the natural fallow was intermediate (Table 1.3). Maize P uptake increased proportional to maize production. Considering the fact that mineral P fertilization also doubled maize yields (and P uptake) and P thus appears to be the primary limiting nutrient, the yield increase after incorporation of fallow biomass may be attributed to improved P availability. However, an interaction with other nutrients, especially N, cannot be excluded. For example, the addition of inorganic N in the pot experiment in chapter 3 increased plant P uptake (Table 3.2). Thus, the generally higher availability of N in MCF than in COM (Table 1.6) may have contributed to differences in maize P uptake between the two rotations in the first pot experiment (Table 3.2) as well as in the field.

In addition to nutritional aspects, slightly improved growing conditions due to other functions of soil organic matter (Craswell and Lefroy, 2001) as well as a lower weed and disease or pest pressure after a fallow period cannot be excluded. For the weed biomass, an increase rather than a decrease was observed in previously fallowed plots, suggesting that in this case, the fallow did not function as a weed break, while for pest pressure, the multiplication of nematode populations under several ‘improved’ fallow species is a serious concern (Desaeger and Rao, 2001).

The continuation of the trial for a total of five fallow seasons gave an example of the complexity of agroecosystems. The productivity of the crotalaria fallow decreased steadily due to pest problems (Figure 1.1), illustrating that there is a danger that ‘haste makes waste’ when trying to improve crop rotations. Any new management system should be tested carefully for several years before promoting it to farmers. This may be even more so when introducing species which are not indigenous and often have a too narrow genetic base to resist pests and diseases.

Certainly, no simple solutions exist to the problem of low soil fertility under conditions of low external inputs in the tropics. A regular fallow break appears to be required to halt losses of soil organic matter (Figure 1.2). However, the yield improvement after fallow biomass incorporation may not amount to more than a compensation of the yield forgone during the fallow season, especially when maize production during the short rainy season is relatively high. In the field experiment, the yield level of the long rainy seasons was reached during 2 out of 5 short rainy seasons in COM (Table 1.3).
Although the risk for pests and diseases is not automatically reduced by increasing plant diversity in agroforestry systems (Schroth et al., 2000), a diversification of fallow species as well as crops is probably beneficial. This applies also to the search for maize cultivars adapted to low P availability. In view of the absence of net P uptake in the pot experiments as well as the low production of the Kenyan hybrid maize without P fertilization in the field, improvements by plant breeding and selection may be possible. However, any increase in maize production through a greater mobilization of soil P, either by the crop or by the fallow, will also increase P outputs and may eventually reduce P availability. Considering the relatively large amount of total P in the soils in western Kenya, this may be buffered for some time. To prevent a degradation of the system, however, the P balance should be leveled out by additions of mineral P fertilizer.

In the field experiment, P fertilization increased maize P uptake in the maize-crotalaria rotation from 8 to 17 kg P ha$^{-1}$ (Figure 5.2). It also increased outputs of N (Table 1.4). The input of N into the system through BNF by a legume fallow may balance the increased output of N after P fertilization, but without addition of N fertilizer, the overall N balance remains negative. Interestingly, even the non-leguminous natural fallow increased N contents in the topsoil compared to continuous maize (Figure 1.3). Most probably, this is due to recycling of N from lower soil layers to the topsoil, but non-symbiotic N$_2$ fixation may also play a role, the extent of which is poorly quantified. The lack of response of fallow productivity to P fertilization (Table 1.2) does not necessarily mean that improved fallow species will not benefit from an increase in available P$_i$. Indeed, the dry matter production of several improved fallow species, including crotalaria, was enhanced by the addition of P fertilizer in pot experiments with soils from western Kenya (Benjamin Kibor, personal communication). In the field, the availability of P$_i$ to the fallows was probably not improved due to the limited incorporation of fertilizers applied to the previous maize crop, in combination with low water availability during the fallow season which resulted in fallow P uptake from lower soil layers (Figure 1.3).

To avoid negative nutrient balances and achieve sufficient yields, it is mandatory that mineral fertilizers become affordable to farmers in marginal regions such as western Kenya. However, the application of mineral fertilizers has to be combined with practices that sustain soil organic matter and prevent soil degradation.
General discussion and conclusions 127

Figure 5.1: Schematic presentation of general processes in soil P dynamics

Figure 5.2: P dynamics in a maize-legume fallow rotation on a Ferralsol in western Kenya. Boxes show amounts of P in plant and soil compartments in kg ha\(^{-1}\), with values after 4 annual applications of 50 kg P ha\(^{-1}\) (as TSP) in brackets. Dotted arrows and numbers in circles show the relative distribution of fertilizer and plant residue P (in % of applied) in the soil, as concluded from the recovery of \(^{33}\)P in the various pools after 51 days (note that the percentages recovered in \(P_{\text{ex}}\) and in the NaOH-pools may partly overlap). Controlling factors on microbial dynamics in italics, processes not studied in this work in brackets. The interrupted arrow shows changes in the community composition when C availability is high. Note also the relationship between the pool sizes of available P\(_{i}\) and microbial P.
Changes in soil P pools

While the fallows caused a shift towards organic and microbial P (Table 1.7), the addition of P fertilizer improved the availability of $P_i$ (Table 1.6) without affecting levels of organic and microbial P. The resulting pairs of soils from a given crop rotation with similar soil organic matter contents at different levels of $P_i$ created an interesting context for the study of P transformations.

The quantification of soil $P_o$ poses analytical problems, especially in highly weathered soils. With sequential extraction, the amount of $P_o$ in each fraction is determined as the difference between $P_i$ and $P_{tot}$, which introduces a source of error (Tiessen and Moir, 1993). Changes in $P_o$ fractions are often not detectable against the large amount present, while any smaller P pool which is determined directly may be more sensitive. Previous studies showed that the amount of P contained in macro-organic matter (> 250 μm) was elevated 2-4 months after incorporation of fallow biomass (Maroko et al., 1999; Smestad et al., 2002), with an increase of 1-3 mg P kg$^{-1}$ compared to continuous maize. In the first study cited, a decrease in this fraction occurred during the subsequent cropping period, suggesting that $P_o$ was mineralized and that this fraction represented a labile source of P.

In this thesis, differences in soil $P_o$ between crop rotations were assessed with the ignition method (Saunders and Williams, 1955), knowing that it may overestimate $P_o$ in highly weathered soils (Condron et al., 1990). While the accuracy of this method is certainly limited, the extraction with 0.1 M NaOH (chapter 4) recovered 79% of the $P_o$ determined with the ignition method. According to these two methods, at least 31-39% of $P_{tot}$ in these soils is in organic form (Figure 5.2), but the nature of the 50% of $P_{tot}$ that remained unextracted with the ignition method is even more obscure than that of the extracted portion. The recovery of 80% of carrier-free applied $^{33}$P with only 40% of $P_{tot}$ 10-51 days after addition of the label (Table 4.6) suggests that a large proportion of $P_{tot}$ participates at very low rates in isotopic exchange processes, and may be in quite stable inorganic or organic forms.

The main focus of this project was on the small but active, living compartment of soil $P_o$, i.e. microbial P. Figure 5.2 shows that in a maize-legume fallow rotation, the microbial biomass contains at least 10 kg P ha$^{-1}$, determined as $P_{hex}$. This is 5-10 times more than $P_{resin}$ and $E_{1min}$ in a soil without P fertilization. In the field, up to 84% of the
variation in $P_{\text{hex}}$ was explained by a multiple linear regression with the factors $C_{\text{tot}}$ and $N_{\text{min}}$ ($n = 24$). In an incubation experiment, the microbial biomass took up substantial amounts of $P$ within two days after amendment of soils with glucose ± $N$ (Figure 1.4), and at high levels of $C$ and $N$ availability, only the initial extent of $P$ immobilization was increased by $P$ fertilization (Figure 2.3). It was concluded that the level of microbial $P$ in this soil is limited by $C$ and $N$ rather than $P$ availability.

While the amount of microbial biomass is usually closely related to levels of soil organic matter (Wardle, 1992), $P$ availability may affect rates of decomposition. From their comparison of neighboring tropical forest sites on a highly weathered Oxisol and a fertile Mollisol (on alluvial material), Cleveland et al. (2002) concluded that microorganisms in highly weathered soils may be $P$ limited. They observed that the decomposition of added substrates (glutamate, salicylate, glucose) was accelerated by simultaneous additions of $P$, with the effect being much stronger in the highly weathered soil. In the absence of $C$ amendments, however, high additions of $P$ (250 mg kg$^{-1}$) were required to increase soil respiration in the Oxisol, with the effect becoming non-significant after 2 weeks. Thus, these data suggest a co-limitation by $C$ and $P$ rather than a primary $P$ limitation of microbial processes.

In this thesis, the effect of increased $P_i$ availability resulting from $P$ fertilization in the field and not from fresh additions of $P_i$ was studied, except for the low addition in chapter 4 (6 mg $P$ kg$^{-1}$) which did not increase soil respiration (Table 4.4). While the decomposition of added glucose and especially cellulose proceeded more rapidly in $+P$ treatments (Figure 2.2), soil respiration of non-amended soils was not increased by three annual applications of 50 kg $P$ ha$^{-1}$ each (Table 1.7). It was concluded that except at high levels of $C$ availability, $P$ fertilization does not affect microbial growth and activity. Presumably, microorganisms in this soil are well adapted to low $P$ availability. They may have effective $P$ uptake systems, produce phosphatases to increase mineralization rates, and substitute $P$-containing molecules with $P$-free equivalents, e.g. phospholipids with glycolipids (Harder and Dijkhuizen, 1983).
**P immobilization and re-mineralization**

The extent of microbial P immobilization differed widely when glucose and cellulose were added at the same rate of C (Figure 2.3). Thus, C availability to microorganisms is a function of the quantity and the quality of C. In the case of plant residue amendment, the additional P uptake by the microbial biomass within one week was closely related to the amount of water-soluble C added to a given soil ($r^2 = 0.91-0.93$; Table 2.3 and Figure 2.6). Glucose and water-soluble C from residues may have a large short-term effect on microbial dynamics because they diffuse more easily in soil than cellulose and non-soluble C, and because rapidly growing microorganisms such as gram-negative bacteria will proliferate on them. The structural components of plant residues, which may support other organisms such as fungi, increase the microbial biomass to a lower extent but for longer times (Figure 2.6). Besides the effect of substrate quality, the extent of P immobilization was also higher in MCF than in COM, which may be due to the greater initial size as well as the different composition of the microbial biomass.

The microbial P compartment thus increases from 10 kg P ha$^{-1}$ at the end of a fallow phase (Figure 5.2) to between 13 and 20 kg ha$^{-1}$ after incorporation of fallow biomass, as concluded from the increase in $P_{\text{hex}}$ after addition of crotalaria residues equivalent to 5 and 8 Mg dry matter ha$^{-1}$ (chapter 4 and 2, respectively). More than half of this increase may be derived from the soil and not from the added residue (Figure 4.3), illustrating the competition of the microbial biomass with plants for soil P. Indeed, available $P_i$ generally decreased when microbial P increased (Figure 2.4 and 2.6), but the measured decrease in $P_{\text{resin}}$ was smaller than the concomitant increase in $P_{\text{hex}}$. A partial buffering from other P pools was also suggested by a lower specific activity in $P_{\text{resin}}$ after addition of the labeled residue than after carrier-free addition of $^{33}$P (Figure 4.2).

The large increase in $P_{\text{hex}}$ after addition of either glucose or crotalaria residue was followed by a rapid decline (Figure 2.3a, 2.6a), during which $P_{\text{resin}}$ increased. Similar to immobilization, the extent of re-mineralization may increase with the initial level of microbial biomass (Figure 2.6a, 2.7a). In addition to C availability, abiotic stress such as desiccation and rapid rewetting presumably controls the size of the microbial biomass. Microorganisms respond to drying of soils by accumulating intracellular solutes and secreting polysaccharides which form an extracellular sheet. Both strategies may
decrease water losses to the more concentrated surrounding solution (Potts, 1994). Rapid rewetting of soils results then in a release of accumulated solutes, or in cell lysis if the turgor becomes too large (Kieft et al., 1987). Drying and rewetting processes may be quite common in the topsoil under the high temperatures and intense solar radiation in the tropics, and soil microorganisms should therefore be adapted to it. However, certain groups such as gram-negative bacteria which are lacking protective cell walls may be quite susceptible to changes in soil water potential (Halverson et al., 2000), with the evolutionary strategy to rely on the rapid multiplication of a few surviving cells rather than to increase the number of survivors. Drying of soil a week after addition of plant material decreased the newly formed biomass relatively more than drying four weeks after addition, suggesting that fast growing microorganisms are more susceptible to desiccation than those with slower growth rates (van Gestel et al., 1993). These examples concern primarily the interaction of drying and rewetting with levels of labile C in the soil. However, a direct effect on P dynamics has also been shown: Simulated rainfall on moist soil cores sampled from a tropical forest during the rainy season increased P immobilization, whereas it resulted in P release from dry season soil cores (Campo et al., 1998).

Presumably, abiotic stress can decrease the microbial P pool to below the level determined by substrate availability, with a corresponding increase in available P$_i$ (Figure 5.2). Such hypothesized larger fluctuations in the microbial biomass in the field than at constant soil moisture in laboratory incubations would make microbial P the most dynamic P compartment in the soil. This may be especially important for P cycling in a highly weathered soil for which the equilibrium distribution of P between the soil solution and the solid phase is almost completely on the side of the latter.

Even the flux in and out of the microbial biomass at a constant size due to cell metabolism may contribute to P cycling, but attempts to quantify this flux are confronted with problems. In this study, rapid microbial uptake (within one day) of 8 and 13% of carrier-free applied $^{33}$P was observed in COM and MCF, respectively (Figure 4.2). In both soils, a lower percentage (4 and 9%, respectively) was recovered after ten days, which remained unchanged thereafter, although the specific activity in P$_{resin}$ continued to decrease. The same applied even in the case of a net change in P$_{hex}$ after plant residue addition, where the specific activity in P$_{hex}$ showed no clear time
trend (COM) or remained similar from 10 days onwards (MCF). Thus, P fluxes between pools could not be detected beyond 10 days. To relate microbial P fluxes and fluctuations to P availability for plants is then even more difficult. This would especially apply to the study of flush effects, where any decrease in water availability in the pot experiment stresses not only soil microorganisms but also the plant. The study of seasonal patterns of P pools in the field, however, suggests that fluctuations in microbial and organic P are important for meeting the plant demand for P (Chen et al., 2003).

**Microbial synthesis of soil organic P**

Plant residues represent a mixture of C, P<sub>i</sub> and P<sub>o</sub> as well as other nutrients. Often, more than half of the total P in plant material is in inorganic form (Daroub et al., 2000; Salas et al., 2003), as was observed in this study (Table 2.3, 4.2). However, rapid hydrolysis of P<sub>o</sub> by plant enzymes during extraction of residues (Martin and Cunningham, 1973) cannot be excluded. In the soil, P<sub>o</sub> from plant material may be stabilized through sorption of phosphate groups, or together with soil organic matter, i.e. through the carbon moiety (Stewart and Tiessen, 1987). In the study by Salas et al. (2003), about 25% of the total P added with plant residues was recovered in macro-organic matter directly after addition, decreasing to the level of the non-amended controls within five days. Thereafter, the amount of P in macro-organic matter increased again, suggesting that most of it may be derived from microbial immobilization rather than originating from plant material.

In the present study, 20% of the label applied with the plant residue was recovered in NaOH-P<sub>o</sub> (Figure 5.2), and this proportion remained unchanged between 10 and 51 days after amendment (Figure 4.4). In the case of residue addition, a distinction between P<sub>o</sub> originating from the plant material and microbial synthesis, respectively, is not possible. In the case of carrier-free labeling of soil IEP and addition of labeled P<sub>e</sub>, however, the recovery of up to 6% of applied label in P<sub>o</sub> can only be a result of microbial synthesis.

Earlier studies found an increase in P<sub>o</sub> after amendment of soils with cellulose (Chauhan et al., 1981) and during incubation of leaf litter (Mueller-Harvey and Wild, 1986). The existence of stereoisomers of inositol phosphates in soil that do not occur in plants or animals also points to the participation of microorganisms in determining chemical forms of soil P<sub>o</sub> (Turner et al., 2002), either through direct synthesis, or through
transformation of isomers originating from plants through epimerization (changes between stereoisomers), the physiological reason for which remains to be shown.

Mineralization of soil organic P

The rates of basal mineralization of soil $P_0$ in a maize-legume fallow rotation could not be determined. Using plants as indicators gave variable results: The first pot experiment with non-residue amended soils receiving a P-free nutrient solution indicated higher amounts of P in maize shoots growing in soil MCF than in COM (Table 3.2), but the second pot experiment did not confirm this (Table 4.8). Here, a limitation occurred in MCF which was attributed to a nutrient other than P and N. A general problem with the two pot experiments was the absence of significant P uptake above the amount contained in the seedlings at transplanting. Other pot trials with highly weathered soils have also indicated none or very little uptake of P above the amount contained in the seeds of *Lolium* and *Agrostis* species (Truong and Pichot, 1976; Bühler et al., 2003) or found significant correlations between plant dry matter and the amount of P in the seed of different rice cultivars (Hedley et al., 1994). The limited soil volume and time frame of pot experiments, especially in combination with the use of radioisotopes, reduces the usefulness of approaches to use plants as indicators for P availability when they take up little or no P. Even in the field, the amount of P in the above-ground maize biomass at harvest did not surpass the 20-fold amount of seed P during 6 out of 11 growing seasons in COM–P.

The rapid uptake of applied $^{33}$P into $P_{hex}$ after carrier-free labeling of soil IEP, amounting to a maximum of 13% in this study after one day (Figure 4.2), may represent a source of error for the determination of $P_0$ mineralization rates with the isotopic dilution method of Oehl et al. (2001b). During the stirring connected with labeling of moist soil, C sources may become available which activate microorganisms, whereas in the batch experiment, soil is shaken with water overnight before $^{33}$P is introduced. Microbial uptake of $^{33}$P at the end of the batch experiment could be investigated with the use of liquid fumigation and compared to the recovery of $^{33}$P in $P_{hex}$ of incubated samples after the same period of time. The influence of basal $P_0$ mineralization on measured specific activities in the soil solution (or any other P pool for which the baseline of physicochemical processes has been established) should be small at 60 or
100 min, so that extrapolated and measured specific activities should agree, unless the potential activation of microorganisms induces a sudden release of unlabeled P. In the study by Oehl et al. (2001b), the difference between extrapolated and measured specific activities was not yet significant after one day and increased until 10 days, giving confidence that the observed isotopic dilution was not caused by different conditions during soil labeling and in the batch experiment. However, only 4% of applied $^{33}$P was recovered in the microbial biomass after 5 days in the same soil (Oehl et al., 2001a).

Using the daily soil respiration rate of 5-6 mg C kg$^{-1}$ in fallow soils (Table 1.7) and a C:P$_o$ ratio of 94 (as concluded from the possibly overestimated amount of soil P$_o$ determined with the ignition method) results in the daily hydrolysis of 0.06 mg P kg$^{-1}$ from P$_o$ driven by the search for energy, part of which may be taken up by microorganisms. However, this is a very rough estimate, as no CO$_2$ is released during the enzymatic hydrolysis of P$_o$. Besides, too little information exists for these soils about the quality and quantity of P$_o$ substrates available for mineralization and about the use efficiency of the microbial biomass. Nevertheless, it can be stated that the measurable changes in P$_{hex}$ during immobilization and re-mineralization cycles are at least an order of magnitude greater than the release of P$_o$ connected with the decomposition of soil organic C. Such microbial fluctuations may thus influence P availability more than basal mineralization of soil P$_o$, but in contrast to the latter, this can mean replenishment as well as withdrawal of available P.$^i$

**Composition and functions of microbial communities**

The analysis of PLFA profiles indicated that in addition to the higher amount of microbial biomass in MCF than in COM, the composition of the community also differed between crop rotations (Figure 2.1). The relative abundance of fungi and gram-negative bacteria increased and that of gram-positive organisms decreased in MCF compared to COM, as concluded from different indicator fatty acids for specific organism groups (Table 2.4). The greater proportion of fungi may have accelerated the degradation of added cellulose (Figure 2.2), but this cannot be separated from size-of-biomass effects. Witter and Kanal (1998) cautioned that microbial substrate utilization differed significantly between soils from a field experiment with different levels of microbial C when glucose was added at a constant rate, but not when the amount of
glucose added was adjusted to twice the amount of microbial C. This agrees with the observation of a higher microbial P uptake in MCF than in COM after plant residue addition in the order of 6 g dry matter kg\(^{-1}\) (Figure 2.6), but not when 3.3 g kg\(^{-1}\) were added (Figure 4.2).

For any discussion on the “role of the infinitely small”, the great diversity of soil microorganisms, estimated to amount to thousands of species in a few grams of soil, contrasts with the small proportion (<1%) that is culturable and can be studied directly (Torsvik et al., 1994). It has been questioned whether a detailed knowledge about the actors is required to understand processes at the ecosystem level, as simple models can usually predict processes such as decomposition without including organism dynamics (Andrén et al., 1999). In the case of nutrient cycling and to optimize synchrony between nutrient availability and plant demand, it may however be important to identify keystone species with a disproportionate effect on process rates, and specialists, i.e. microorganisms with functional traits that are not redundant by occurring in a great number of species. For the soil microbial biomass, fumigation-extraction methods do not even allow a reliable estimation of the size of the extracted ‘black box’ because of the uncertainty connected with conversion factors. Therefore, new methods based on molecular biology have the potential to improve our understanding of the composition and functions of soil microorganisms, including the spatial heterogeneity of soil processes. This knowledge may then result in better soil management from the agronomic as well as environmental perspective.
Main conclusions and outlook

The incorporation of fallow biomass was shown to enhance the biological processes of soil P dynamics. Maize yields were also increased, but less reliably than by the application of mineral P fertilizer, which increased levels of available $P_i$ without a significant effect on the biological processes in the soil. The microbial biomass is suggested to represent the most dynamic P compartment in a highly weathered soil. It was shown that the presence of labile C results in pronounced patterns of P immobilization and re-mineralization. While rates of basal $P_o$ mineralization could not be determined, there was evidence for microbial synthesis of soil $P_o$. It is hypothesized that flush effects due to abiotic stress will amplify microbial P dynamics in the field.

In addition to necessary changes in the political and socioeconomic context, research and extension in regions such as western Kenya should continue to strive for the following ideal situation:

Besides maize varieties that are well adapted to the local soil constraints, a range of other crops is grown and marketable due to improved infrastructure as well as demand. Mineral fertilizers are affordable and used efficiently through placement and good timing. Nutrient cycles are closed as much as possible by returning animal manure to the field and reducing soil losses due to erosion. Legume fallows grown during the short rainy season are well adapted to the environment, producing a large amount of biomass, which mobilizes a great amount of soil P, stimulates the microbial biomass, makes a significant input of N through BNF, and maintains levels of soil organic matter. The processes increasing P cycling are understood in such a way that they can be manipulated to increase the productivity of the system, e.g. by strategic additions of $P_i$ during periods of immobilization, by synchronizing P re-mineralization with plant P demand, or by stimulating or depressing certain groups of microorganisms. As a result, the nourishment, health, education, and wealth of the people are greatly improved, while at the same time, adverse effects on the environment are minimized.
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Effect of P and N availability on changes in microbial P after plant residue addition

Figure A1: Changes in $P_{\text{hex}}$ and $P_{\text{resin}}$ after addition of crotalaria and maize stover at 2.5 g C kg$^{-1}$ to soils COM±P without (-N) or with (+N) simultaneous addition of NH$_4$NO$_3$ at 0.25 g N kg$^{-1}$; bars above and below symbols for $P_{\text{hex}}$ and $P_{\text{resin}}$, respectively, show HSD$_{0.05}$ for dates at which significant differences between treatments occurred according to ANOVA with the factors P fertilization, N addition and their interaction; dotted symbols indicate non-significant changes compared to the non-amended controls (in which no significant effects of P fertilization on $P_{\text{hex}}$ or of N addition on $P_{\text{hex}}$ or $P_{\text{resin}}$ were observed, not shown).
Batch experiment with anion-exchange resin membranes

Objective
To detect isotopic dilution due to \( P_0 \) mineralization in \( P_{\text{resin}} \), a baseline of the physicochemical processes has to be established for \( P_{\text{resin}} \) against which isotopic dilution could be detected in incubated samples.

Methods
A pre-incubated soil sample (25% water content) from the treatment COM–P sampled in 2000 (chapter 1) was used. The decrease in radioactivity a) in the soil solution and b) on anion-exchange resin membranes (BDH #55164, 31 mm x 20 mm) saturated with \( \text{HCO}_3^- \) was determined at various times up to 100 min after introduction of a known amount of radioactivity. Moist soil (equivalent to 10 and 4 g dry soil for a) and b), respectively) was shaken overnight with \( \text{H}_2\text{O}_{\text{dest}} \) in a soil:solution-ratio of 1:10 (w:v) on an overhead shaker. Subsequently, samples were stirred on a magnetic plate or shaken on a horizontal shaker for the determination in the soil solution and \( P_{\text{resin}} \), respectively. At \( t = 0 \), a known amount of radioactivity (R) was introduced into the system. For the determination in the soil solution, the procedure was as described in Bühler et al. (2003), while Figure A2 shows the procedure for the method with resins.

Figure A2: Schematic presentation of the batch experiment of isotopic exchange with resin membranes
After introduction of \( R \) (approx. 15 kBq g\(^{-1}\) soil), the resins were added after different times (1 resin membrane g\(^{-1}\) soil) of up to 100 min. Three extraction periods were tested, removing the resins after 1, 4 and 16 h, respectively, followed by immediate rinsing with \( H_2O_{dest} \) and elution with 0.5 \( M \) HCl. The radioactivity in the soil solution and in the eluate was determined using a liquid scintillation counter (Packard 2500 TR) with 5 ml Packard Ultima Gold scintillation liquid per 1 ml of sample. In order to achieve complete recovery of standard additions of \(^{33}\text{P}\), a dilution with \( H_2O_{dest} \) (1:4) was required in the case of HCl eluates.

**Results**

For periods of isotopic exchange of up to 100 min, the decrease in radioactivity remaining in the soil solution (Figure A3) followed a power function, where the coefficient and the exponent resemble the parameters \( r_1/R \) and \( n \) in the formula given by Fardeau (1993), respectively. As a first approximation, a similar function appeared to describe the decrease in radioactivity extracted with resin membranes (Figure A4). The fraction of the total introduced radioactivity recovered with resins that were added after 1 min increased from 0.15 to 0.41, when the extraction period increased from 1 to 16 h. At the same time, the value of the exponent decreased, while the amount of \( P_{resin} \) increased from 0.2 to 1.7 mg P kg\(^{-1}\).

Further tests with the extraction period of 1 h indicated that for the soil solution, the decrease in radioactivity in the soil solution for periods of isotopic exchange of up to 1 week was well described by the complete formula given by Fardeau (1993). For \( P_{resin} \), however, a different function would have to be established.

**Conclusions**

It may be possible to establish the baseline describing the decrease in the specific activity due to physicochemical processes also for other pools than the soil solution, such as \( P_{resin} \). This would allow the detection of isotopic dilution due to \( P_0 \) mineralization in this pool.
Figure A3: Decrease in radioactivity in the soil solution of soil COM–P ($n = 3$)

![Graph showing decrease in radioactivity over time for soil COM–P.]

$y = 0.02x^{-0.49}$

$r^2 = 1.00$

Figure A4: Decrease in radioactivity recovered on resins with extraction for a) 1h, b) 4h, and c) 16h and amount of $P_{\text{resin}}$ (soil COM–P, $n = 3$)

**a) resin 1h**

$y = 0.15x^{-0.22}$

$r^2 = 0.97$

$P_{\text{resin}}$ (mg P kg$^{-1}$):

0.185 (SD ± 0.031)

**b) resin 4h**

$y = 0.25x^{-0.12}$

$r^2 = 0.81$

$P_{\text{resin}}$ (mg P kg$^{-1}$):

0.504 (SD ± 0.033)

**c) resin 16h**

$y = 0.41x^{-0.07}$

$r^2 = 0.89$

$P_{\text{resin}}$ (mg P kg$^{-1}$):

1.665 (SD ± 0.169)
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