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Author(s):
Liebisch, Frank

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Plant and soil indicators to assess the phosphorus nutrition status of agricultural grasslands

A dissertation submitted to the
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presented by

Frank Liebisch
Dipl.-Ing. agr. – Universität Hannover
born 27.06.1978
citizen of Germany
(European Union)

accepted on the recommendation of

Prof. Dr. E. Frossard, examiner
Dr. E.K. Bünemann, co-examiner
Dr. C. Jouany, co-examiner

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Pictures:

Front: grasslands in Watt,

Les Verrièrres and Baldegg,

by myself,

Rear: *Quadrifolium spec*., Eschikon,

by Monika Welc.

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Summary

Phosphorus (P) is an essential element for life and as such a key element for agriculture where it is applied as fertilizer to obtain high yields and quality of crops. When appearing in surplus P can have detrimal effects on the environment (e.g. eutrophication of surface waters). At the same time, the world reserves of rock phosphate are limited. Thus, an efficient P management in agriculture is necessary for economic and ecological reasons.

Grassland is a major land use type in agriculture. The interpretation of plant nutrient concentrations in grassland samples is complex due to variation in botanical composition, changing concentrations during growth and interactions between nutrients. Although soil P analysis is routinely used to estimate plant available P in the soil, the actual P availability is often ill defined by these tests. This is particularly true for grassland soils where organic matter is accumulated in the topsoil and microbial activity is high. To avoid yield losses and P accumulation it is necessary to characterize the transition between deficient, sufficient and surplus P nutrition status as precisely as possible.

The aim of the project was to evaluate a set of plant and soil indicators to accurately identify the P nutrition status of agricultural permanent grasslands. We studied three typical permanent grassland types managed at different harvest intensities with no (P0), sufficient (P1) or surplus P (P2) inputs since 5 to 20 years. The grasslands were located at Les Verièrres (V), Watt (W) and Baldegg (B). During the growing season of 2008, plants were sampled at vegetative stage and differentiated into botanical fractions (grasses, legumes and forbs) at each harvest and soils were sampled once (0 to 5, 5 to 10, 10 to 20 and 20 to 40 cm). In 2009 we conducted a study on the seasonal dynamics of P availability and its drivers in one of these grasslands (W).

For the plant P indicators we measured plant P, nitrogen (N) and potassium (K) concentrations in the aboveground biomass of grasses, legumes and forbs. Subsequently we evaluated P concentration, N:P and K:P ratios, the P nutrition index (PNI) and the P index derived from the diagnosis recommendation integrated system (DRIS P). Grasses were the main botanical fraction (52-88%) in all swards. The identification of P nutrition status was found to be more precise in grasses than in bulk sward samples. The critical P concentration in grasses below which yield was reduced was 2.1 to 2.3 mg g^{-1} while the maximum yield response was found at 3.5 mg P g^{-1} indicating a surplus P nutrition status. The PNI showed the clearest differentiation between deficient, sufficient or surplus fertilizer inputs. To identify

nutrient co-limitation additional nutrient indicators need to be considered. In principle, N:P, K:P and DRIS are all applicable, but we suggest to refine the values used for interpretation of these indicators for nutrient diagnosis in agricultural grasslands.

For the soil indicators, we aimed to establish thresholds for soil P availability using yield and plant P nutrition status response curves. We used three soil P indicators based on different principles: an extraction method (P_{ox}), an isotope exchange method (E_{1min}) and an ion sink method (P_{res}). In the 0 to 5 cm soil layer, the P0, P1 and P2 treatments were significantly differentiated by the three soil P indicators. In 20 to 40 cm depth only P2 fertilized treatments were significantly higher than P1 and P0 for the three soil P indicators. For the plant indicators yield response, P concentration and PNI in grasses, relationships to P_{ox}, E_{1min} and P_{res} were stronger for combined sites than for specific locations. For these relationships we established exponential functions with a maximum. We applied thresholds identified in the previous study to determine the soil P availability classes deficient, sufficient and surplus for plant growth. We identified low and high thresholds of 290 and 510 for P_{ox}, of 5 and 14 for E_{1min}, and of 4.5 and 14 mg P kg^{-1} soil for P_{res}. Among the three P indicators P_{res} had the strongest coefficients of determination with grass P concentration and PNI, thus it reflected sward P nutrition status better than P_{ox} or E_{1min}.

In the next study we applied the same approach to the two Swiss standard methods for soil P extraction with CO_2 saturated water (P_{CO2}) and ammonium-acetate EDTA (P_{AAE}). For P_{CO2} a combined use of the locations was not appropriate. To propose thresholds we had to consider the soil with high P buffer capacity in V separately from the soils in W and B which had a similar buffer capacity. For P_{AAE} the interpretation without additional soil information seemed reasonable for the investigated locations. For both P_{CO2} and P_{AAE}, the soil P availability in the 0 to 5 cm soil layer was underestimated by the soil P classification scheme.

Finally, we studied the effect of different P fertilizers on the turnover of P through the microbial biomass and its consequences for P availability. We selected three P input treatments (no P (P0), mineral P (Pmin) and organic P (Porg) fertilization) in the permanent grassland in W. During the growing season of 2009, soil samples were taken frequently. Available P was measured as resin-extractable P (P_{res}) and microbial P (P_{mic}) was determined by hexanol fumigation with simultaneous extraction by anion exchange resin membranes, without application of correction factors. Consistent increases of P_{mic} in the three treatments were observed after the summer and autumn harvests when plant growth rate was low. Reduced P_{mic} and increased P_{res} were observed after dry periods. From the observed

fluctuations in P_{mic} we calculated P_{mic} turnover and fluxes. The turnover rate of P in the microbial biomass tended to be higher in P0 than in Pmin and Porg. The fluxes of P through the microbial biomass and the mean P_{mic} stocks of P in the microbial biomass were two to three times higher than plant P uptake. The potential of P_{mic} to withhold available P during times of high P demand of plants or under dry conditions indicated a competition for available P, particularly when P availability is low.

Both plant and soil P indicators are valuable tools for the diagnosis of P nutrition status in permanent grasslands. With the thresholds established in this study, the investigated indicators can be interpreted for permanent grasslands under field conditions. For plant indicators we recommend to use the PNI in the grass fraction assisted by other nutrient indicators to exclude limitation of or co-limitation with other nutrients. The establishment of soil P status classes was improved by the combined use of plant yield and P nutrition status indicators. The most reliable soil P indicator in this study was P_{res} in field moist soil. However, the thresholds developed for P_{ox}, E_{1min}, P_{CO2} and P_{AAE} identify the three soil P status classes as well. Whereas plant indicators differentiated most precisely between a deficient and sufficient status, soil analysis was more precise to differentiate a sufficient from a surplus P nutrition status. Thus, we propose a strategy for a combined use of both indicators for sward P diagnosis. In view of the strong effects of P_{mic} and climatic conditions on P availability and P cycling under field conditions, these interactions should be addressed by further research.

Zusammenfassung

Phosphor (P) ist ein lebenswichtiges Element und als solches von grundlegender Bedeutung für die Landwirtschaft, wo es als Dünger appliziert wird, um hohe Erträge und Qualitäten der Kulturen zu gewährleisten. Ein übermässiges Vorkommen von P kann sich aber negativ auf die Umwelt auswirken (z.B. Eutrophierung von Gewässern). Gleichzeitig sind die weltweiten Reserven für Rohphosphat begrenzt. Deshalb ist ein effizientes Management von P in der Landwirtschaft sowohl aus wirtschaftlichen als auch aus ökologischen Gründen notwendig.

Grasland ist eine der wichtigsten Nutzungsformen in der Landwirtschaft. Die Interpretation von Nährstoffkonzentrationen in den Pflanzen des Graslandes ist aufgrund hoher Variation in der botanischen Zusammensetzung, sich ändernder Konzentrationen während des Wachstums und der Interaktionen zwischen den Nährstoffen sehr komplex. Die P-Analyse im Boden wird heute routinemässig angewandt, definiert aber die Pflanzenverfügbarkeit des P im Boden oft

ungenau. Das trifft besonders für Graslandböden zu, wo der Oberboden hohe Gehalte an organischer Substanz aufweist und die mikrobielle Aktivität hoch ist. Um Ertragsverluste und die Akkumulation von P im Boden zu vermeiden, ist es notwendig, die Übergänge zwischen mangelhafter, ausreichender und übermässiger P-Versorgung genau zu charakterisieren.

Das Ziel dieser Arbeit war die Bewertung von Pflanzen- und Bodenindikatoren zur genauen Identifikation des P-Versorgungszustandes im landwirtschaftlich genutzten Grasland. Wir untersuchten drei typische Graslandstandorte, welche seit 5 bis 20 Jahren mit jeweils unterschiedlichen Nutzungsintensitäten (2, 3 und 5 Schnitte pro Jahr) und Düngeverfahren (ohne P (P0), ausreichend (P1) und übermässig (P2)) bewirtschaftet wurden. Die Standorte waren Les Verièrres (V), Watt (W) und Baldegg (B). Im Jahr 2008 wurden die Wiesenpflanzen und Böden beprobt. Die Pflanzenproben wurden im vegetativen Stadium genommen und nach botanischen Fraktionen getrennt (Gräser, Leguminosen und Kräuter). Die Böden wurden in den Tiefen 0 bis 5, 5 bis 10, 10 bis 20 und 20 bis 40 cm beprobt. 2009 führten wir eine Studie zur jahreszeitlichen Dynamik der P-Verfügbarkeit und deren Einflussfaktoren auf dem Standort in W durch.

Für die Pflanzenstudie haben wir die P-, Stickstoff- (N) und Kalium- (K) Konzentrationen in den Pflanzenproben gemessen, um den P-Status des Graslandes anhand der folgenden Indikatoren zu bestimmen: P-Konzentration, N:P- und N:K-Verhältnisse, den P-Ernährungsindex (PNI) und den P-Index aus dem Integrierten Diagnose-und Empfehlungssystem (DRIS P). Die Gräser waren die grösste botanische Fraktion (durchschnittlich 52 bis 88%) in allen untersuchten Standorten. Die Fraktion der Gräser war zudem präziser in der P-Status-Bestimmung als die Gesamtproben. Wir fanden die kritische P-Konzentration, unter der der Ertrag signifikant reduziert war, bei 2.1 bis 2.3 mg P g^{-1}. Der maximale Ertrag war bei 3.5 mg P g^{-1} erreicht. Über diesem Wert ist also eine übermässige P-Versorgung angezeigt. Der PNI zeigte die deutlichste Unterscheidung zwischen P-Mangel, ausreichender und übermässiger P-Versorgung. Um eine Ko-Limitierung mit anderen Nährstoffen festzustellen, ist es nötig, mehrere Indikatoren einzubeziehen. Dafür besonders geeignet sind N:P, K:P und DRIS. Obwohl im Prinzip verwendbar, empfehlen wir für diese Indikatoren eine Verbesserung der Interpretationsgrundlage für die Nährstoffdiagnose im Grasland.

In der Bodenindikatorstudie verfolgten wir das Ziel, mit Hilfe von Ertrag und P-Status der Pflanzen, Grenzwerte für die P-Verfügbarkeit im Boden zu klassifizieren. In einem ersten

Ansatz untersuchten wir drei Boden-P-Indikatoren, welche auf unterschiedlichen Prinzipien basieren: eine chemische Extraktion (P_{ox}), eine Isotopenaustausch-Methode (E_{1min}) und eine Ionensenke-Methode (P_{res}). In der obersten Bodenschicht (0 bis 5 cm) wurden die Düngeverfahren P0, P1 und P2 durch die drei Indikatoren signifikant unterschieden. In 20 bis 40 cm Tiefe waren die Werte der P2-Verfahren höher als die Werte von P0 und P1, welche sich nicht unterschieden. Die Beziehungen zwischen den drei Bodenindikatoren und den Pflanzenindikatoren Ertrag, P-Konzentration und PNI waren am stärksten, wenn die drei Standorte kombiniert wurden. Für diese Beziehungen etablierten wir exponentielle Funktionen mit einem Maximum. Folgend benutzten wir die Grenzwerte des P-Status der Pflanzen aus der vorhergegangenen Studie, um die P-Verfügbarkeit im Boden in mangelhaft, ausreichend und übermässig zu klassifizieren. Wir identifizierten jeweils den unteren und oberen Grenzwert für P_{ox} bei 290 und 510 mg P kg^{-1}, für E_{1min} bei 5 und 14 mg P kg^{-1} und für P_{res} bei 4.5 und 14 mg P kg^{-1} Boden. Unter den drei Bodenindikatoren hatte P_{res} die stärkste Beziehung mit den P-Status-Indikatoren der Pflanzen.

Im nächsten Schritt benutzten wir den gleichen Ansatz für die Standardmethoden der Bodenanalyse in der Schweiz. Das sind die Extraktion von P mit CO_2-gesättigtem Wasser (P_{CO2}) und Ammoniumazetat-EDTA (P_{AAE}). Für P_{CO2} war die Beziehung zu den Pflanzenindikatoren für kombinierte Standorte nicht aussagekräftig. Um Grenzwerte zu finden, unterteilten wir deshalb die Standorte nach Boden-P-Pufferkapazität, welche in V höher war als in W und B. Für P_{AAE} war die Interpretation der Beziehungen über alle drei Standorte ohne zusätzliche Bodenparameter aussagekräftig. Im offiziellen Klassifizierungsschema wurde die P-Verfügbarkeit im Boden für beide Methoden zu gering eingestuft.

In der letzten Studie untersuchten wir den Einfluss verschiedener P-Dünger auf den P-Umsatz in der mikrobiellen Biomasse im Boden und die damit verbundenen Konsequenzen für die P-Verfügbarkeit. Auf dem Standort in W (3 Schnitte pro Jahr) wählten wir dafür die folgenden drei P-Düngeverfahren aus: ohne P (P0), mineralisch (Pmin) und organisch (Porg). Während der Vegetationsperiode 2009 wurden die Böden und Pflanzen regelmässig beprobt. Die P-Verfügbarkeit wurde mit Anionenaustauschharzen gemessen (P_{res}). Das mikrobielle P (P_{mic}) wurde mit der gleichen Methode und einer simultanen Hexanolfumigation ohne Korrekturfaktor bestimmt. Wir fanden über alle drei Düngeverfahren einen einheitlichen Anstieg von P_{mic} nach dem Sommer- und Herbstschnitt, als die Pflanzenwachstumsrate niedrig war. Reduziertes P_{mic} und gleichzeitig erhöhtes P_{res} wurde nach Trockenperioden

beobachtet. Aus den gemessenen Fluktuationen von P_{mic} berechneten wir P_{mic}-Umsatz und P_{mic}-Flüsse. Die P-Umsatzrate der mikrobiellen Biomasse war in P0 tendenziell höher als in Pmin und Porg. Die P_{mic}-Flüsse durch die mikrobielle Biomasse und die mittleren P_{mic}-Vorräte im Boden waren zwei bis dreimal grösser als die P-Aufnahme durch die Pflanzen während der Vegetationsperiode 2009. Das Potenzial von $P_{mic,}$ während erhöhtem Pflanzen-P-Bedarf oder Trockenperioden verfügbares P zurückzuhalten, deutet auf eine Konkurrenz zwischen Pflanzen und Mikroben um das verfügbare P hin, besonders wenn dieses gering ist.

Pflanzen- und Bodenindikatoren sind wertvolle Werkzeuge für die Diagnose des P-Status im Dauergrasland. Mit den hier identifizierten Grenzwerten können Daten aus dem Feld nun verlässlich für die untersuchten P-Indikatoren interpretiert werden. Als Pflanzen-P-Indikator empfehlen wir den PNI in der Grasfraktion, unterstützt von weiteren Indikatoren, um eine Limitierung durch andere Nährstoffe auszuschliessen. Die Identifizierung der Boden-P-Versorgungsklassen wurde durch die Kombination von Ertrags- und Pflanzen-P-Indikatoren verbessert. In unseren Studien erwies sich P_{res} als der verlässlichste Bodenindikator für die P-Verfügbarkeit. Nichtsdestotrotz können die hier identifizierten Grenzwerte für P_{ox}, E_{1min}, P_{CO2} und P_{AAE} verwendet werden um die P-Verfügbarkeit des Bodens zu bestimmen. Während Pflanzenindikatoren den Grenzwert zwischen Mangel und ausreichender Versorgung besser beschreiben, sind die Bodenindikatoren für die Unterscheidung von ausreichender und übermässiger Versorgung zu bevorzugen. Deshalb schlagen wir eine Kombination beider Indikatoren für eine verlässliche P-Status-Diagnose im Grasland vor. Die starken Effekte von P_{mic} und den klimatischen Bedingungen auf die P-Verfügbarkeit unter Feldbedingungen sollten durch weitere Studien tiefergehend untersucht werden.

1. General introduction

1.1. The phosphorus dilemma

Phosphorus (P) is an essential element for all living organisms. As such it is an economical factor for agricultural production to maintain yields and quality of crops. Production of P fertilizers is mainly based on mining of rock phosphate which is a finite resource. Its worldwide production is estimated to peak in 20-100 years (Cordell et al., 2009; Van Vuuren et al., 2010). Agriculture (ca. 89%) and the adjacent food industry (1-2%) are the main consumers of P worldwide (Schröder et al., 2010). Thus, agriculture plays a key role for a sustainable management of this resource.

In addition, P strongly impacts the environment when appearing in surplus. Eutrophication of surface waters has been linked to P inputs to fresh water (Carpenter, 2008) and salt water (Diaz and Rosenberg, 2008; Sundareshwar et al., 2003). P losses from agriculture have been identified as the major source of P in surface waters (Tiessen et al., 2011). In addition, the accumulation of P has been related to the decline of biodiversity in marine (Todd et al., 2010) and terrestrial (Klaudisová et al., 2009; Wassen et al., 2005) ecosystems.

During industrial times, human activities have altered the natural P cycle on local and global scales. Whereas P generally accumulates in agricultural soils in industrial countries (Lavelle et al., 2005; Schröder et al., 2010) it limits agricultural production in regions with highly weathered soils (e.g. Sub-Saharan Africa). In industrial countries and on a global scale agriculture has been identified as the main driver of the leakiness of the contemporary P cycle (Tiessen et al., 2011). P losses from agricultural soils are higher from soils where P has accumulated (Preedy et al., 2001).

As a consequence of declining P resources, the world wide P demand and environmental impacts, an increased efficiency of P management in agriculture is necessary (Schröder et al., 2010). Nutrient use efficiency in agriculture is often considered as output per area divided by input per area and is high in western agriculture (Schröder et al., 2010). But such considerations do not include P losses or additional costs caused by environmental impacts (e.g. aeration of eutrophicated lakes). Measures to improve P use efficiency in agriculture encompass plant breeding, recycling of P within the farm and from human wastes (e.g. sewage, slaughter wastes),

a better management of soil and fertilizer P and the use of beneficial organisms to enhance P accessibility and uptake. Hereafter, plant P uptake is defined as the amount of P exported from the field with the harvested above ground biomass. Measures to improve P management include 1) integrated use of plant and soil P indicators to identify the P nutrition status of a system (Breuer et al., 2003), 2) identification of nutrient imbalances and determination of exact fertilizer value of organic fertilizers, facilitating the combined use of mineral and organic fertilizers to reduce such imbalances (Schröder, 2005), 3) field specific identification of plant P demand and subsequently adapted fertilizer recommendation and application (Betteridge et al., 2008) and 4) critical re-evaluation of soil P classes for fertilizer recommendation (Römer, 2009). Thus, to identify and evaluate potential measures to improve P management in agriculture and a better understanding of processes and factors affecting P availability is necessary.

1.2. Phosphorus cycling and availability

The available P pool is defined as the amount of P that can be released to the soil solution during a period of time that is relevant for plant growth (Holford, 1997). Although P from the soil solution is taken up by plants as inorganic P (P_i), a recent study suggests that organic soil P can considerably contribute indirectly to plant P nutrition in the short term (Steffens et al., 2010). The P availability in the soil is affected by abiotic and biotic factors (Frossard et al., 2000). The main abiotic factors are 1. sorption and desorption and 2. precipitation and dissolution of P (Figure 1.1). Biotic processes affecting P availability are 1. mineralization and immobilization of P into microbial or other organic forms and 2. the P uptake and root exudation of plants. For agricultural systems the balance between P input by fertilization and P export from the field is also important for P availability. Particularly the biotic factors are also affected by climatic conditions such as precipitation and temperature (plants and microbes) and irradiation (plant growth). Under field conditions the above described factors and processes are strongly interrelated and often variable in time and space.

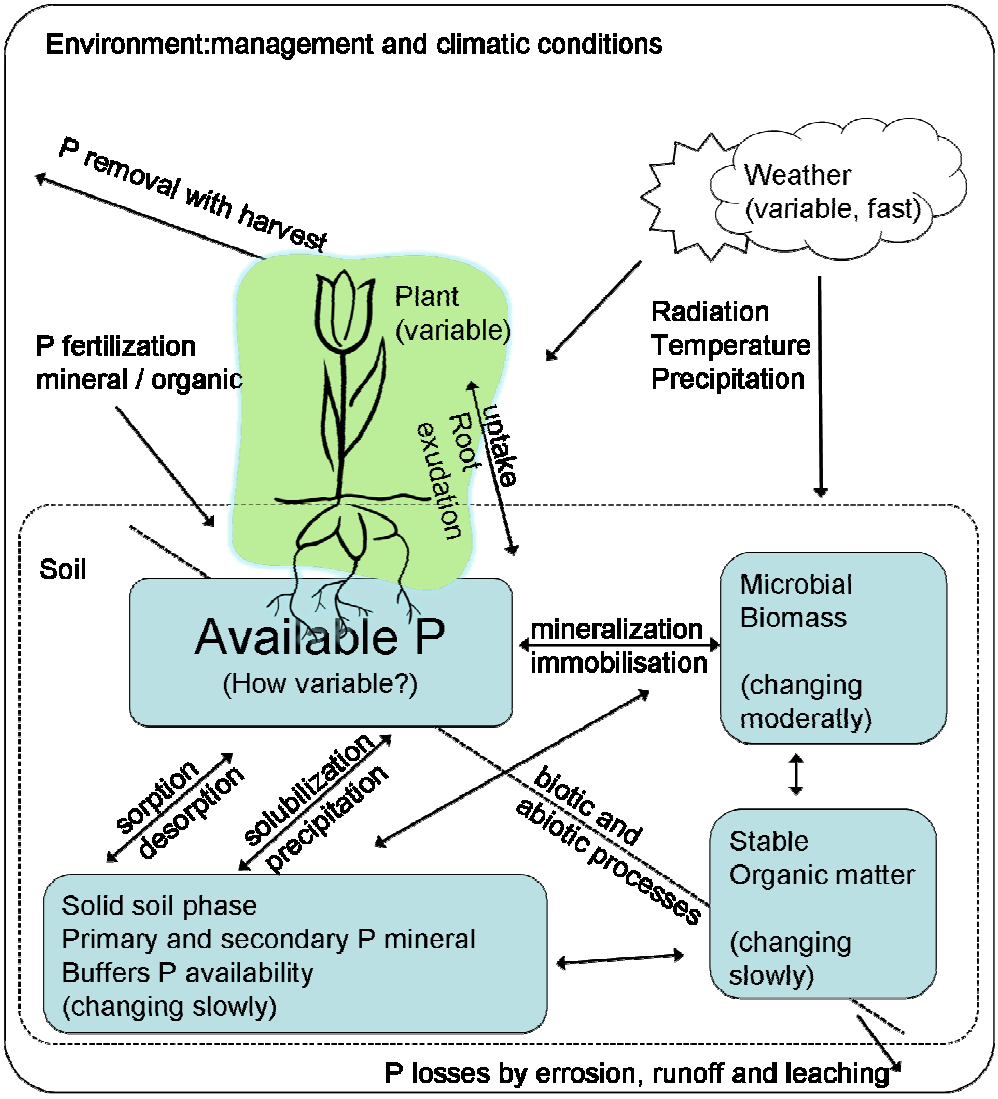

Figure 1. 1: Selected pools and processes affecting P availability in the soil. In brackets an indication of how variable a certain pool is during the season. Modified from Frossard et al. (2000).

1.3. Grassland – a challenge for P management

In addition to forage production, agricultural grasslands fulfill many other functions such as soil protection, biodiversity conservation and as areas for recreation (Gibson, 2009). Higher nutrient inputs and cutting frequency during agricultural intensification in the second half of the 20th century increased yields and quality of forage for livestock production (Kapfer, 2010). This intensification was largely at the expense of plant diversity in agricultural grasslands (Dietl, 1995) and was accompanied by P accumulation in soils (Haas et al., 2001). The loss of plant diversity was mainly caused by high cutting frequencies and nutrient inputs which boosted high yielding and vegetative reproducing species. The P accumulation was often a result of surplus

manure and slurry disposal. In contrast, species-rich grasslands are managed at low intensity, with lower nutrient inputs and less frequent harvesting. They produce lower yields and quality of forage, but they are of high ecological value. Therefore, they are nowadays supported by governmental subsidies in the European Union (Hopkins, 2000) and in Switzerland (Cornaz et al., 2005).

In Europe, P is still accumulating in agricultural soils, particularly under grassland in areas supporting high livestock density (Schröder et al., 2010). In a recent study Binder et al. (2009) showed that Switzerland is a net importer of P and that the highest flows and stocks of P are related to agriculture and human wastes. Eutrophication of water bodies has also been linked particularly to accumulation of P in grasslands and increased P losses from grasslands (Haygarth et al., 2005; Watson et al., 2007). In Switzerland, P accumulation in agricultural soils has been observed as well (Spiess, 1999). Other studies in Switzerland have observed P losses from grasslands (Braun et al., 2001; Stamm et al., 1998) and linked eutrophication of lakes to agricultural P inputs (Gächter et al., 1996; Stadelmann et al., 2002).

Grasslands cover about two thirds of the agricultural area in Europe (Peeters, 2009) and in Switzerland (BfS, 2007). Thus, an improved P fertilizer use in grasslands may have a large impact on overall P use efficiency in agriculture. Compared to arable land, grasslands have a high plant biodiversity and may differ strongly in botanical composition, management, soil and environmental conditions. Thus, the precise estimation of P fertilizer need is difficult. Therefore, widely applicable and reliable indicators for the P nutrition status of grasslands are needed.

To minimize P inputs while maintaining high yields of the herbage an integrated nutrient management is needed (Frossard et al., 2009; Monaghan et al., 2007). The P removed with the harvest needs to be replenished by fertilization to maintain P fertility in the soil. Under P deficient conditions it might be necessary to fertilize P in surplus to increase the P availability in the soil and to achieve higher yields and herbage P concentrations. When yields are at maximum, however, fertilization in surplus to plant demand results in P accumulation in the soil. Such P accumulation reflects an inefficient fertilizer use and results in the detrimental environmental impacts described above. Very high soil P contents can be decreased by means of P export from the soil by above ground plant biomass removal without P resupply by fertilization (P mining), but this might need a long time (Koopmans et al., 2004; Schärer et al., 2007). Because of

the complexity of grassland ecosystems it is difficult to foresee how changes in nutrient inputs will affect grassland productivity and nutrient concentrations in the biomass. To avoid yield losses and P accumulation it is necessary to characterize the transition between deficient, sufficient and surplus P nutrition status as precisely as possible.

1.4. Identification of P nutrition status

The P nutrition status is defined as P deficient when plant growth is significantly lower than the expected yield for a given system and the soil cannot deliver sufficient amounts of P to satisfy plant P demand (see Figure 3.1). In such a situation P fertilization will increase yields and soil P availability. At a sufficient P nutrition status plant yield fulfils the yield expectation and a surplus P fertilization would increase soil P availability but not the plant yield. The surplus P nutrition status defines a situation where expected yields can be reached despite lower or no P fertilization. P enrichment of the soil is defined as a situation when P fertilization has no yield response but may still result in increasing plant P contents. In the case of P accumulation no change of plant P content can be observed when P is added. Both enriched and accumulated soil P status reflect an inefficient P fertilizer use, but accumulated soils may also act as a P source for the environment because maximum plant P uptake is reached.

1.5. The tools: general overview and principles

1.5.1. Plant analysis

Plant tissue analysis is a direct measure of plant nutrient status. It integrates all sources and forms of P which were available for the plant, including uptake from different soil depths and changes over time. However, plant analysis cannot predict long term P availability (Marschner, 1995). Plant analysis has been suggested as a “standalone” tool as well as to assist soil analysis for diagnosis of nutrient status and for fertilizer recommendation (Breuer et al., 2006). Besides it is used to differentiate nutrient from pest and pathogen problems.

From a methodological point of view, plant P concentrations are generally determined after total digestion of plant tissue or by dry incineration followed by acid digestion.

Subsequently, P can be measured using colorimetrical methods such as malachite green (Ohno and Zibilske, 1991) or inductively coupled plasma (ICP) spectrometry. In permanent grasslands, plant analysis is mostly done on bulk samples taken at early vegetative growth or harvest (Flisch et al., 2009; Mackay et al., 1995). Only for scientific purposes specific species such as perennial ryegrass (*Lolium perenne*) or white clover (*Trifolium repens*), botanical fractions or specific parts of the plant are analyzed.

Measured P concentrations are interpreted using critical P concentrations or ranges that indicate sufficient P supply (see Table A.1). Combined nutrient indicators are used to reflect the interaction of P with other elements e.g. nitrogen (N) and potassium (K). Such indicators are the nutrient ratios (N:P and K:P) and the P nutrition index (PNI). The PNI is calculated using a critical curve developed for optimal P concentrations in relation to sward N concentration (Duru and Thélier-Huché, 1997). It was established for ryegrass dominated swards and later confirmed for other grass species (Bélanger and Richards, 1999). In France it is used to determine the P nutrition status of grasslands and to recommend amounts of P fertilization (Jouany, personal communication). A multi-element integration approach is the diagnosis and recommendation integrated system (DRIS) developed by Beaufils (1973). The DRIS method identifies plant nutrient imbalances using many nutrients whose ratios are related to norm ratios. The norm ratios reflect conditions when none of the included nutrients is limiting or in surplus and when high yields are obtained. The resulting DRIS indices are ranked according to their relative importance (Walworth and Sumner, 1987). Bailey et al. (1997a; 1997b; 2000) applied DRIS to perennial ryegrass (*Lolium perenne*) and perennial ryegrass-rich grasslands. Today it is used in Northern Ireland for decision support of sward nutrient diagnosis and fertilizer recommendation (Bailey, personal communication).

The interpretation of plant analysis in grasslands is constrained by high variation in botanical composition, sward age (Daccord et al., 2007) and interactions with other nutrients such as N and K (Osaki et al., 2003). For example, indicators integrating N are affected by legume abundance since legumes have generally higher N concentrations than grasses and other plants. Thus, the legume abundance needs to be considered when the PNI is used in mixed stands (Jouany et al., 2004). The DRIS method has been proposed to overcome the constraints by sward age to some extent (Bailey et al., 2000),

but the interpretation for calcium and magnesium is still unclear (Bailey et al., 1997a). Due to the complexity of grassland P nutrition, ill-defined critical values and optimal ranges of concentrations and ratios, today plant P analysis in grasslands is mostly used to calibrate or assist soil P analysis for fertilizer recommendation (Bergmann, 1993).

1.5.2 Soil analysis

For soil analysis a wide variety of chemical extractions are used (Neyroud and Lischer, 2003). They are used mostly on dried and sieved soil and are comparatively rapid and cheap. All methods are used to characterize P availability to plants and thus predict P fertilizer response. Extracting agents can be water, diluted acids or complexing agents. In principle, two classes of soil P extractions can be differentiated (Marschner, 1995). Weak extractions, e.g. with water or bicarbonate, reflect mainly easily available P, whereas strong extractions, e.g. acids or ammonium oxalate, extract more P than is plant available in one season.

The P availability in soils is better characterized by the concept reviewed by Holford (1997) which uses the three factors intensity, quantity and buffer capacity. The intensity is the activity of P_i in the soil solution and usually given in mg P L^{-1}. The quantity (mg P kg^{-1}) represents the reserves of P in the soil that can be released to the soil solution with time. The buffer capacity of a soil describes the ability of the soil to keep the intensity constant when the quantity changes, for example due to plant P uptake or P fertilization (Figure 1.1).

The degree of P saturation (DPS) and the isotopic exchange kinetics (IEK) take these factors better into account than simple extraction methods (Frossard and Sinaj, 1997; Schoumans, 2000). The DPS was initially developed to estimate the risk of P losses from soils (van der Zee et al., 1987) based on an oxalate extraction of P. The IEK is used to describe the transfer of P from the solid soil phase to the soil solution and vice versa. Both methods are used today to estimate the P availability in soils (Bell et al., 2005a; Morel et al., 2000; Tran et al., 1988).

Other approaches to estimate P availability in soils mimic plant P uptake as a P sink (Myers et al., 2005). Anion exchange resins or iron coated filter papers are used as P sinks. Sink approaches have been shown to be less affected by soil properties and were

often better correlated to plant P uptake (Qian et al., 1992). Thus, they are suggested to reflect plant P availability in the soil better than many routine soil tests.

A drawback of chemical extraction is that often not all available P is extracted while variable amounts of non-available P might be extracted (Demaria et al., 2005). Although most of the P extractions are mutually correlated they often extract different forms of P and thus should not be correlated to classify plant P availability for another extraction. This should always be tested in plant growth experiments. Soil analysis does not provide information about actual P mobility (diffusion) in the soil under field conditions and changes of available P during the growing season. By sampling a given soil layer the actual rooting depth is neglected when P availability is estimated. By drying the soils as it is done for most soil analyses the actual availability in the soil is changed, for example by release of P from the microbial biomass or changed reactivity of binding sites for P in the soil (Schärer, 2003; Turner and Haygarth, 2001). In grasslands, P availability is particularly difficult to predict by soil analysis (Herlihy et al., 2006). Often the interpretation is constrained by high organic matter contents, biological activity and accumulation of nutrients in the topsoil. Differences in management and environmental conditions also affect short and long-term P availability, uptake and use by plants.

Soil analysis methods have often been standardized using fertilizer response experiments on the main soil types for a given region or country. For the interpretation, soil analysis results were mostly calibrated to yield response or plant P uptake. Subsequently the soil P analysis was categorized into classes according to probability of crop response. The established soil P status classes can therefore often only be used for a certain range of soils and conditions. In many European countries a 5 step classification system is used (e.g. A to E) for interpretation (Flisch et al., 2009; Kerschberger et al., 1997; MAFF, 2000). The intermediate class (C) represents a sufficient P nutrition status and A and E reflect a deficient and accumulated P nutrition status, respectively. However, today it is argued that thresholds identifying these classes are too high and thus P recommendation often exceeds actual plant demand (Römer, 2009; Schröder et al., 2010). Consequently a re-evaluation or adjustment of the interpretation schemes is asked for.

In Switzerland the extractions with CO_2 saturated water (P_{CO2}) and with ammonium oxalate EDTA (P_{AAE}) are used as standard methods to determine the P nutrition status

of soils (FAL et al., 1996). Whereas P_{CO2} reflects the P in the soil solution, P_{AAE} is a stronger extraction (Flisch et al., 2009). Both extracts are interpreted in relation to clay content and organic matter in the soil. Whereas P_{CO2} was found to underestimate rapidly exchangeable P, P_{AAE} was found to extract non-available P forms from the soil (Demaria et al., 2005). As described above an adjustment of the interpretation scheme for these methods is required (Frossard et al., 2004; Gallet, 2001).

1.5.3 Plant versus soil analysis

Both plant and soil analysis have advantages and disadvantages and provide different information. Plant P analysis reflects only the actual P nutrition status as result of the past time of growth. Soil analysis reflects the potential P availability in medium or long term. Thus, neither plant nor soil analysis can be substituted and their complementary use results in a better diagnosis of P nutrition status, particularly for grasslands. In Switzerland, average sward P uptake derived from long term fertilizer experiments is used to recommend amounts for P fertilizer application (Flisch et al., 2009). But plant analysis is not yet (frequently) used as a diagnostic tool to assess the nutritional P nutrition status of crops in agri- or horticulture.

1.5.4. Environmental factors

As described above (Figure1.1), the P availability is also affected by environmental factors. Such environmental factors can be weather and climate conditions, soil parental material, soil structure, slope or exposition in the landscape and many more. For this study we will focus on weather and climate factors.

Plant growth and P uptake are affected by temperature, soil moisture and light intensity (Marschner, 1995). Plant growth in temperate grasslands usually stops at temperatures below 5 and above 40°C (Whitehead, 2000). High light intensity increases plant growth and thus P demand when soil moisture is favourable. The diffusion of P in the soil and plant uptake of P are reduced when soil moisture is low (Dunham and Nye, 1976) and subsequently plant growth is diminished.

The biomass productivity curve of temperate grasslands reflects the interaction of climatic factors (Gibson, 2009). Lowest productivity is found in winter when

temperatures are low. Peaks of sward productivity occur in spring and in late summer when growth conditions are good. The highest productivity is generally found in spring because light intensity is high and soil moisture is sufficient. During summer a growth reduction due to high temperature and low soil moisture is often observed.

Processes such as mineralization and immobilisation of P are also affected by soil moisture and temperature. Due to the high biological activity and below ground biomass in grasslands, such processes are often of greater importance than under arable land (Brookes et al., 1984). The microbial P (P_{mic}) turnover, an important driver for P cycling is also affected by environmental factors (Chen et al., 2003; Oberson and Joner, 2005). Given the strong influence of environmental factors on plant P demand and P availability, climate data can support the interpretation of plant and soil P indicators.

1.6. Aim and hypothesis

The aim of the project was to develop and evaluate a set of soil and plant indicators to accurately identify the P nutrition status of agricultural permanent grasslands. We wanted to assess the effect of different long term P inputs on soil P availability, plant P uptake and herbage production. Therefore, the indicators should reflect at least the three P nutrition status classes deficient, sufficient and surplus P nutrition. Additionally they should be applicable for different grassland types.

We hypothesize that these indicators can be used to 1) improve diagnosis of P nutrition status and 2) better understand processes affecting P availability in grasslands. Both a precise P nutrition status diagnosis and a better understanding of P availability in grasslands may help to improve P fertilizer use efficiency and limit P losses to the environment.

1.7. Structure of the thesis

Chapter 2

The aim of this chapter was to evaluate the ability of several plant P indicators (P concentration, N:P and P:K ratio, PNI and DRIS P) to assess the sward P nutrition status in permanent grasslands. To assess the effect of botanical composition on the P

nutrition status identification we analyzed the three botanical fractions grasses, legumes and forbs separately in three different grassland types (see 1.8). To identify deficient, sufficient and surplus P nutrition of the swards we evaluated the plant P indicators according to relative yield responses as caused by differences in long term fertilizer application. Finally we wanted to propose critical values and norms to improve the interpretation of plant P analysis in permanent grasslands.

Chapter 3

In chapter three we aimed at establishing thresholds for P availability for three soil P indicators: P extracted with ammonium oxalate (P_{ox}), P exchangeable in one minute (E_{1min}) and resin extractable (P_{res}). The three methods were chosen as examples for a chemical extraction, an isotope approach and a sink method to estimate available P in the soil. To identify soil P classes we established exponential functions with a maximum for the relationship between the three soil P indicators and plant yield, P concentration and PNI in grasses (chapter 2). To classify the soil P indicators into deficient, sufficient and surplus P we used the thresholds for plant P indicators derived in chapter two.

Chapter 4

In this chapter we applied the same procedure as in chapter three to the Swiss routine soil P extractions with CO_2-saturated water (P_{CO2}) and ammonium-acetate EDTA (P_{AAE}). The obtained thresholds were compared with the actual Swiss soil P classification (Flisch et al., 2009).

Chapter 5

In chapter five we investigated the dynamics of plant available and microbial P (P_{mic}) in a grassland managed at low intensity during one season (in 2009). Our main objective was to measure P_{mic}, P_{mic}-fluxes and turnover as affected by P fertilization and to reveal interactions with plant available P under field conditions. Additionally we wanted to deduce effects of weather and plant growth on both P_{res} and P_{mic}. Therefore, we selected three types of P fertilization: no P (P0), mineral P (Pmin) and organic P (Porg) and sampled soils and plants on a regular basis. Weather conditions and soil moisture were monitored.

1.8. Study sites and experimental setup

1.8.1 Selection criteria

Given the research question we searched for permanent grasslands with different management intensity and long term P input. Management intensities practiced in Switzerland range from extensively used grasslands with one harvest per season to very intensively managed grasslands with six harvests per year (Flisch et al., 2009). We focused on fertilized agricultural grasslands. A prerequisite for our study was that the long term "P input treatments" resulted in conditions of deficient, sufficient and surplus P availability for plant growth. Agricultural grasslands are fertilized to avoid yield losses and Gallet et al. (2003a) found no yield decline in 6 out of 7 field experiments even nine years after omission of P fertilization. Thus, long term fertilizer experiments including P treatments were the first choice for our study. However, we did not find a long term fertilizer experiment for intensively managed grasslands. For intensively managed grasslands we selected on-farm grasslands with different soil P status (see table 4.1 for Swiss soil P classification).

1.8.2. Selected study sites

We selected three locations with different cutting frequency, fertilizer input and environmental conditions: 1) north of Les Verrières (V) in the Jura mountains, 2) Watt (W) near Zürich and 3) in the eastern area of the Lake Baldegg (B) watershed (Figure 1.2). The locations V and W are long-term fertilizer trials with different rates of N, P and K applications since 1992 and 1996, respectively. Location B is an agricultural region which has been dominated by intensive livestock production and grassland management for forage production with high application rates of animal manure during the last decades. The fertilization rates for the three locations are shown in detail in table 2.3.

The two fertilizer trials in V and W were managed by the research institutions Agroscope Changins-Wädenswil and Agroscope Reckenholz-Tänikon, respectively. The on-farm swards were managed by farmers, with similar fertilization and cutting regime for the last 5 to 20 years. In 2008, the swards in V, W and B were cut 2, 3 and 5

times, respectively. The management intensity of each grassland (Table 1.1.) is defined by altitude and number of harvests per year (Flisch et al., 2009).

Table 1.1: Geographical location and selected management indicators for the investigated grasslands

Location	**Geographical location**	**Altitude** (m.a.s.l)	**Management intensity**[a]	**Number of harvests**	**Managed by (since)**
Les Verrières	46°56'18"N, 6°27'38"E	1150	semi intensive	2	Agroscope Changins-Wädenswil (1992)
Watt	47°26'44"N, 8°29'32"E	500	low intensity	3	Agroscope Reckenholz-Tänikon (1996)
Baldegg	47°11'55"N, 8°31'01"E	560-780	intensive	5-6	Farmers (5-20 years)

We selected four treatments with increasing mineral P fertilization in V. In W, five mineral fertilizer treatments and an organically fertilized treatment from another experiment located on the same site were selected. In B we chose two grasslands which were fertilized as recommended and two which were over-fertilized with P. The particular types and rates of fertilizers used for each site and treatment are described in detail in chapter 2. For the labeling of the treatments, the following nomenclature is used throughout the whole document: N, P and K stand for the three nutrients followed by 0, 0.5, 1 and 2 for no, less, close to equal and more fertilizer applied than recommended by Flisch et al. (2009). The suffix org and org/min stands for the use of slurry and combined mineral fertilizer and slurry application. In chapter 5 the labeling was simplified (see section 5.2) because only three P fertilizer treatments in W were investigated in depth.

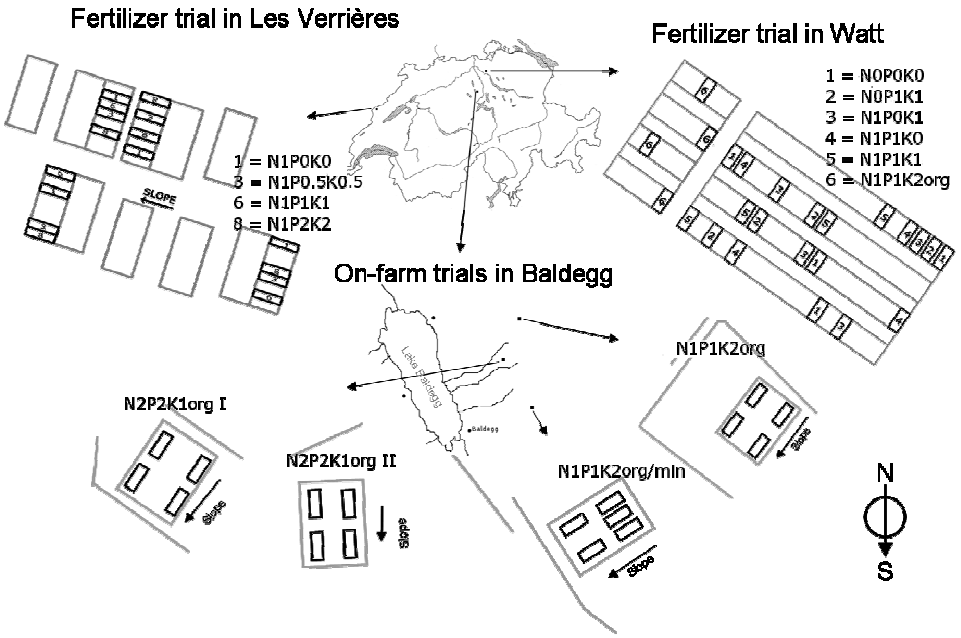

Figure 1.2: Location of experimental fields in Switzerland and experimental setup of the plots

The experimental setup included at least four plots for each treatment to reflect variation in the field. In the fertilizer trials V and W, the allocation of the plots was given by the original randomized block design of the experiments (Figure 1.2). On-farm in B, four to five plots of the same size as in the fertilizer trials were arranged as spatial replicates within each investigated grassland (Figure 1.2). The distance between these plots was at least 5 meters.

The two locations W and B are mesic (moderately moist habitat) grasslands characterized by a warm climate, a vegetation period longer than two thirds of the year and a reasonably balanced hydrology (Table 1.2). The precipitation is generally high and relatively evenly distributed during the season. The grasslands in W and B reflect a plant productivity curve during the season which is typical for productive temperate grasslands (Gibson, 2009). The difference in management and annual yield between W and B is mainly a result of the number of harvests and nutrient inputs.

In V a very moist mountainous climate prevails. The average temperature and vegetation period is lower and the annual precipitation is higher than at the other two locations (Table 1.2). Given that the growth period is only about half a year long, a system with two harvests per year reflects already a semi-intensive management. In V the yield and number of harvests is much more restricted by climatic factors than in W and B.

Table 1.2: Ten year-averages (1999 -2009) of temperature, precipitation and vegetation days of the studied locations. Environmental data were recorded by the closest meteorological station, Swiss Meteo Service IdaWeb (La Brevine, Zürich-Affoltern and Lucerne for Les Verrières, Watt and Baldegg, respectively).

Location	**Temperature** (°C)	**Precipitation** (mm yr^{-1})	**Vegetation days**[a] (days yr^{-1})
Les Verrières	5.8	1400	184
Watt	9.8	1077	257
Baldegg	10	1200	256

[a] number of days with daily mean temperature above 5°C

The botanical composition of the grasslands was recorded in May 2007 in W and in April and June 2008 in B and V, respectively (Table 1.3). A species inventory was made for each plot and the species abundance was estimated using soil cover rates (Dietl, 1995). A detailed description can be found in Annex. The three grassland habitats are typical anthropogenically formed grasslands with characteristic vegetation developed under the respective site properties, climate conditions and management intensity.

Les Verrières is a subalpine grassland with fresh to moist hydrology and slightly acid soil reaction. According to Klötzli et al. (2010), the vegetation can be described as a *Festuco-agrostietum* association (a species-rich grassland with 30-50 flowering species). The generally low agronomic value of these sward is based on a short vegetation period which leads to low dry mass production (2 cuts a year) and on the late first cut which produces forage of low quality (low protein and energy but high fiber content).

Watt is a naturally well drained mesic grassland habitat (dry periods possible in summer) with slightly acid soil reaction. The vegetation is a typical *Arrhenaterion elatioris* association (30-40 species). In 2006 a grassland flower mixture was sown in for a biodiversity experiment conducted by O. Huguenin (personal communication) but did not show significant germination in the year of botanical investigation (2007). The agronomic value can be substantial with relatively high dry matter yields and forage quality.

The location in Baldegg is characterized by a warm climate and the grasslands are mesic habitats with high nutrient inputs and 4 to 6 cuts per year. The mean species number in the investigated grasslands is 18-25. The vegetation can be characterized as high yielding *Trifolio-Lolion* association. These grasslands are of high agronomic value because of their high biomass production and generally high forage quality produced by early cutting. The harvested material is used mainly for silage.

Table 1.3: Average number of species and main grass species in the investigated grasslands

Location	**Number of species**				**Main grasses**
	total	grasses	legumes	forbs	
Les Verrières	24-36	6-8	4-6	14-23	*Festuca rubra, Agrostis capillaris* and *Anthoxanthum odoratum*
Watt	21-36	7-10	4-7	10-15	*Holcus lanatus, Arrhenaterum elatius, Anthoxanthum odoratum, Festuca rubra,* and *Trisetum flavescens*
Baldegg	9-20	3-7	1-2	5-11	*Lolium perenne, Lolium multiflorum, Poa pratensis, Alopecurus pratensis* and *Dactylis glomerata*

Chapter 2: Plant phosphorus indicators evaluated in differently managed permanent grasslands

2.1. Abstract

A critical evaluation of phosphorus (P) fertilizer recommendation is necessary for an efficient P management. Plant P analysis gives a P indicator of sward P nutrition status. But the interpretation of nutrient concentrations in grassland samples is complex due to variation in botanical composition, changing concentrations during growth and interactions between nutrients. We studied three typical permanent grassland types in Switzerland that were under different management intensity mostly receiving low, sufficient or high rates of P fertilizer for 5 to 25 years. We measured plant P, nitrogen (N) and potassium (K) concentration in the above ground biomass of grasses, legumes and forbs. We evaluated the following P indicators: P concentration, N:P and K:P ratios, the P nutrition index (PNI) and the P index derived from the diagnosis recommendation integrated system (DRIS P). We observed yield reduction by more than 10% of yield obtained with fertilization according to recommendation in treatments where one or more nutrients were omitted from fertilization. Fertilizer P input higher than recommended did not significantly increase yield. Grasses were with 52-88% of mean above ground dry matter yield the main botanical fraction in all swards. Under P limiting conditions, forbs and legumes had significantly higher P concentrations than grasses. The coefficient of variation of P and K concentrations within treatments was higher in legumes and forbs than in grasses and the proportion of legumes affected the P indicators integrating N. Therefore, P indicators were applied to the grass fraction instead of the bulk sward samples. All P indicators differentiated between P fertilized and non-P fertilized treatments. The PNI showed the clearest differentiation between deficient, sufficient or surplus fertilizer inputs. The critical P concentration in above ground grass biomass sampled at vegetative stage below which yield was reduced was 2.1 to 2.3 mg g^{-1}. Concentrations higher than 3 mg P g^{-1} indicated luxurious consumption. For the precise and correct interpretation of plant P nutrition status in permanent grasslands, we propose a combined use of P indicators in the grass fraction.

2.2. Introduction

Efficient phosphorus (P) management is a prerequisite for a sustainable agriculture. Among other measures to improve P use efficiency in agriculture Römer (2009) and Schröder et al. (2010) suggested a critical evaluation of actual P fertilizer recommendations using current knowledge. Plant P indicators could be used to calibrate soil P analysis or as standalone indicators to determine P fertilizer recommendation (Breuer et al., 2003).

Grasslands cover the majority of the agricultural area in Europe (Peeters, 2009) and in Switzerland (BfS, 2007). P accumulation due to surplus P inputs (Watson et al., 2007) and increased P losses were observed mainly under intensive managed grasslands (Haygarth et al., 2006). Thus, grasslands play a key role for improvement of P use efficiency in agriculture. The agricultural intensification in the second half of the 20th century resulted in increased yields and quality of forage for livestock production, but at the expense of plant diversity in the sward and increased soil P accumulation. Decreasing soil P contents by means of P export by plant biomass (without resupply of P) has been suggested as a measure to decrease P losses (Schärer et al., 2007).

In Switzerland, P fertilizer recommendation for grassland is based on average annual P removal adjusted to the soil available P nutrition status (Flisch et al., 2009). In grasslands nutrient availability and uptake are difficult to predict by soil analysis (Herlihy et al., 2006). Differences in management and environmental conditions affect short and long-term nutrient availability, uptake and use. The interpretation is constrained by high organic matter contents, biological activity, soil diversity and accumulation of nutrients in the topsoil. In contrast, plant tissue analysis accounts for all plant available nutrient sources and is applicable for many mineral nutrients in the same analysis. A drawback is that plant analysis often comes too late to correct an indicated nutrient deficiency in the investigated standing biomass. For permanent grassland, this is of minor importance because diagnosed nutrient deficiencies can be corrected for the next (re-)growth.

Measured P concentrations are interpreted using either critical P concentrations below which dry matter production is limited by P or concentration ranges that indicate sufficient P supply. In case of multi element analysis, nutrient ratios (N:P and K:P) and other combined nutrient indicators were developed to include the interaction of P with other elements. Such indicators are the P nutrition index (PNI, Duru and Thélier-Huché, 1997) and the diagnosis and recommendation integrated system (DRIS, Walworth and Sumner, 1987). The PNI is

calculated using a critical curve developed for optimal P concentrations in relation to sward N concentration. The DRIS method identifies plant nutrient imbalances using many nutrients whose ratios are related to a norm ratio, norm meaning that none of the included nutrients was limiting or in surplus. The resulting DRIS indices are ranked according to their relative importance. Bailey et al. (1997a; 1997b) applied DRIS to perennial ryegrass (*Lolium perenne*) and perennial ryegrass-rich grasslands.

Plant analysis in grasslands is either done on bulk samples (Flisch et al., 2009) or on specific species such as perennial ryegrass or white clover (*Trifolium repens*) as in Høgh-Jensen and Schjoerring (2010). Thus, critical values and optimal ranges of concentrations and ratios are still under discussion because they might differ for species, grassland type and management. In grasslands, the botanical composition and thus nutrient concentrations may vary, making bulk sward analysis difficult to evaluate (Whitehead, 2000). Plant species as well as botanical fractions can differ in nutrient uptake and use efficiency. Legumes in mixed stands with grasses access P and K better under N deficiency than under high N supply as shown by Jouany et al. (2005) for P and by Øgaard and Hansen (2010) for K. Both works resulted in the conclusion that the application of the nutrient indicators PNI and K:N, respectively, was not appropriate on bulk sward samples. Little is known about the effect of forbs on the nutrient concentration in bulk samples, but the high variety of botanical families found in the forb fraction (Osaki et al., 2003) suggests a high variation.

The aim of our study was to evaluate and compare plant P indicators for the identification of the sward P nutrition status in permanent grasslands. Therefore, plant P indicators ranging from simple P concentrations to P in relation to N and K and an indicator integrating several nutrients were investigated over a range of different management intensities and environmental conditions in Switzerland. We analyzed the three botanical fractions grasses, legumes and forbs separately to assess the effect of botanical composition on the evaluation of sward P nutrition status and the different plant P indicators. To our knowledge such a comparison of different plant P indicators in permanent grassland has not yet been done. To identify deficient, sufficient and surplus P nutrition of the swards we evaluated the plant P indicators according to long term fertilizer application and relative yield response. Finally, we aimed at proposing critical values and norms to improve interpretation of plant P analysis in permanent grasslands.

2.3. Material and Methods

2.3.1. Experimental sites

Three locations with different cutting frequency, fertilizer input and environmental conditions were selected for this study: 1) north of Les Verrières (V) in the Jura mountains (46°56’18”N, 6°27’38”E, 1140 m above sea level), 2) Watt (W) near Zürich (47°26’44”N, 8°29’32”E, 500 m above sea level) and 3) in the eastern area of the Lake Baldegg (B) watershed (near 47°11’55”N, 8°31’01”E, 560-780 m above sea level). The locations V and W are long-term fertilizer trials with different rates of N, P and K applications since 1992 and 1996, respectively. Location B is an agricultural region dominated by livestock production and intensive grassland management for forage production with high rates of animal manure applications.

2.3.2. Environmental conditions and botanical composition

Climatic data were obtained from a nearby meteorological station available by services of MeteoSwiss the Federal Office of Meteorology and Climatology (IDAWEB, 2009). Mean annual temperature, precipitation and number of vegetation days of the sites are shown in Table 2.1.

The botanical composition was recorded in May 2008 in W and in April and June 2009 in B and V, respectively. A species inventory was made for each plot and species abundance was estimated using classes of yield proportion and soil cover according to Dietl (1995). The three grassland habitats are typical anthropogenically formed grasslands with characteristic vegetation developed (Table 2.1) under the respective site properties, climate conditions and management.

Table 2.1: Selected environmental and botanical properties of the studied locations. Environmental data was recorded by the closest meteorological station, Swiss Meteo Service IdaWeb (La Brévine, Zürich-Affoltern and Lucerne for Les Verrières, Watt and Baldegg, respectively).

Location	**Temp-erature**[a]	**Precip-itation**[a]	**Vegetation days**[ab]	**Altitude**	**Number of species**				**Main grasses**
	[°C]	[mm yr^{-1}]	[days yr^{-1}]	[m.a.s.l]	total	grasses	legumes	forbs	
Les Verrières	5.8	1400	184	1150	24-36	6-8	4-6	14-23	*Festuca rubra, Agrostis capillaris* and *Anthoxanthum odoratum*
Watt	9.8	1077	257	500	21-36	7-10	4-7	10-15	*Holcus lanatus, Arrhenaterum elatius, Anthoxanthum odoratum, Festuca rubra,* and *Trisetum flavescens*
Baldegg	10	1200	256	560-780	9-20	3-7	1-2	5-11	*Lolium perenne, Lolium multiflorum, Poa pratensis, Alopecurus pratensis* and *Dactylis glomerata*

[a] Ten year average (1999 -2009),

[b] number of days with daily mean temperature above 5°C

The location V is a subalpine mesic grassland. The soil is a slightly acid Cambisol (FAO, 1998) which has low base saturation (<50%). The soil texture in the top 20 cm is a silty loam (Table 2.2). According to Klötzli et al. (2010), the vegetation can be described as a *Festuco-agrostietum* association (species rich grassland with 30-50 angiosperm species). The generally low agronomic value of this sward is based on a short vegetation period resulting in low dry mass production (2 harvests per year) and the late first harvest which produces a low quality forage (low protein and energy but high fiber content). Watt is a well-drained mesic grassland habitat established on a medium deep (50-70 cm) Cambisol (FAO, 1998) with medium base saturation (50-80%). The vegetation is an *Arrhenaterion-elatioris* association with 30-40 angiosperm species. The agronomic value can be substantial with relatively high dry matter yields and forage quality. For purposes of biodiversity conservation, the first harvest cannot take place before 15th of June, leading to minor forage quality of this harvest. The grasslands at B are mesic with high nutrient inputs and 4 to 6 harvests per year used for silage or hay. The soils are Eutric Cambisols (FAO, 1998) with a high base saturation (60-95%). The texture ranges from loamy sand to silty loam (Table2.2). We characterized the vegetation as a high yielding *Trifolio-lolion* association (Klötzli et al., 2010) with 18-25 angiosperm species. These grasslands are of high agronomic value because of their high biomass production with high forage quality produced by harvests at early growth stage.

Table 2.2: Selected soil properties of the experimental grasslands at the investigated locations and treatments. The clay, silt and sand contents of the soils were determined in the 0 to 10 cm soil layer. The pH, total carbon (Ctot), nitrogen (Ntot) phosphorus (Ptot) and P_{CO2} were determined in the 0 to 5 cm soil layer.

Location	Treatment	clay[a]	silt[a]	sand[a]	pH[b]	Ctot[c]	Ntot[c]	P_{CO2}[a]	Ptot[d]
		%				g kg^{-1}		mg kg^{-1}	
Les Verrières (V)	N1P0K0	30	55	15	6.0	39.3	4.0	0.20	565
	N1P0.5K0.5				5.9	39.1	4.1	0.25	688
	N1P1K1				5.8	36.3	3.9	0.39	732
	N1P2K2				5.5	35.7	3.8	0.55	841
Watt (W)	N0P0K0	22	34	44	6.1	28.5	3.0	0.37	580
	N1P0K1				5.5	29.2	3.0	0.34	555
	N1P1K0				6.0	28.8	3.1	1.26	679
	N1P1K1				5.8	30.6	3.1	0.84	658
	N0P1K1				5.7	29.6	3.1	0.86	730
	N1P1K2org				6.8	38.8	4.2	0.96	713
Baldegg (B)	N1P1K2org	26	31	43	6.8	40.1	4.1	4.60	1574
	N1P1K2org/min	21	51	28	6.5	32.4	3.4	1.77	1609
	N2P2K1org I	16	31	53	6.9	40.5	4.2	16.70	2103
	N2P2K1org II	26	51	23	7.5	59.8	4.9	10.70	2505

[a] according to Swiss reference methods in (Agroscope et al., 1996)

[b] In H_2O, ratio soil:H_2O = 1:2.5

[c] measured by CNS analyzer (Thermo Finnigan, Milan, Italy)

[d] extraction with 0.5 M H_2SO_4 after incineration

2.3.3. Management and fertilizer treatments

The two fertilizer trials in V and W were managed by research institutions Agroscope Changins-Wädenswil and Agroscope Reckenholz-Tänikon, respectively. Farmers managed the on farm trials in B. The fertilizer trials in V and W were established in 1992 and 1996, respectively. The on farm swards were fertilized and harvested similar since 20 years and in case of N1P1K2org/min since five years. The swards in V, W and B were cut 2, 3 and 5 times, respectively in 2008 (Table 2.3). For each treatment, four plots of approximately 2 m x 5 m were used. In the fertilizer experiments V and W, the allocation of the plots was given by the original set up of the experiments. On farm in B, four plots of the same size were arranged as spatial replicates within the investigated grassland, with at least 5 meters distance between plots. In one case in B (N1P1K2org/min) a 5^{th} plot was installed because of local inhomogeneity of botanical composition observed during botanical investigation. For the labeling of all treatments the following nomenclature was used: N, P and K stand for the three nutrients followed by 0, 0.5, 1 and 2 for no, less, close to equal and more fertilizer applied than recommended by Flisch et al. (2009). The suffix org and org/min stands for the use of slurry and combined mineral fertilizer and slurry application. The particular rates of fertilization for each site are shown in Table 2.3.

In V four treatments with increasing mineral P and K applications and similar N supply were chosen from a randomized split block experiment. Fertilizers were applied as ammonium nitrate after the first cut, as super-phosphate and potassium salt in spring or in autumn after the last cut. In W, five mineral fertilizer treatments from a randomized block experiment (Huguenin-Elie et al., 2006), and an organically fertilized treatment from another block experiment located on the same site were chosen. Fertilizers were applied as ammonium nitrate (split in three applications: in spring, after the first and after the second cut), super-phosphate and potassium (applied in spring) for mineral N, P and K fertilization, respectively. In the organically fertilized treatment dairy slurry was applied in spring and after the first cut. In B two grasslands were fertilized as recommended and two were over-fertilized with P (Table 2.3). Dairy or pig slurry produced on the respective farm was usually broadcast applied at the onset of the vegetation in spring and after each harvest. In the year of study (2008), there was no spring application of slurry in B. In N1P1K2org/min no slurry was applied after the second harvest, but 27 kg N ha^{-1} was supplied in form of ammonium nitrate.

Table 2. 3: Management, fertilizer treatments and recommended fertilization (Flisch et. al, 2009) of the experimental grasslands at the investigated locations Les Verrieres (V), Watt (W) and Baldegg (B) in 2008. The treatments are labeled using the following nomenclature: V, W or B for the location and N, P and K for the three mineral nutrients followed by 0, 0.5, 1 and 2 for no, less, equal or more fertilizer than recommended.

Management (altitude) and location	**Treatment**	**Fertilizer**	**Cuts**	**Yield indication**	**Fertilization**		
					N	**P**	**K**
			cuts yr^{-1}	t $ha^{-1}yr^{-1}$	kg $ha^{-1}yr^{-1}$		
Medium intensity (1000-1500 m above sea level)							
Recommendation			**2**	**5.0**	**25**	**15**	**79**
Les Verrières	V-N1P0K0	mineral	2		25	0	0
	V-N1P0.5K0.5	mineral	2		25	9	29
	V-N1P1K1	mineral	2		25	17	58
	V-N1P2K2	mineral	2		25	26	116
Low intensity (< 700 m above sea level)							
Recommendation			3	**6.5**	**40**	**17**	**79**
Watt	W-N0P0K0	mineral	3		0	0	0
	W-N1P0K1	mineral	3		45	0	83
	W-N1P1K0	mineral	3		45	17	0
	W-N1P1K1	mineral	3		45	17	83
	W-N0P1K1	mineral	3		0	17	83
	W-N1P1K2org	dairy slurry[a]	3		55	17	102
Intensive (>~700 m above sea level)							
Recommendation			**5**	**11.5**	**130-**	**39**	**228**
Baldegg	B-N1P1K2org	dairy slurry[a]	5		192	31	548
Intensive (<600 m above sea level)							
Recommendation			**5-6**	**13.5**	**150-**	**48**	**280**
Baldegg	B-	dairy slurry[a] +	5		144+27	24	411
	B-N2P2K1org I	pig slurry[a]	5		210	116	256
	B-N2P2K1org II	pig slurry[a]	5		210	116	256

[a] Estimated according to Flisch et. al (2009) assuming 1:1 dilution of slurry by water.

2.3.4. Basic soil characteristics

The soil texture (Table 2.2) of the two fertilizer trials was measured prior to their establishment in 1992. For the on farm soils it was determined prior to this experiment using dried soil sampled taken in 2008. Soil texture was analyzed using the sedimentation method according to Swiss reference methods for soil analysis (FAL et al., 1996). Additionally, we sampled the soil at a depth of 5 cm after the first regular harvest. We took twelve soil cores with an inner diameter of 2.5 cm distributed over each plot and mixed them into one composite sample per plot. The soil was sieved at 2 mm and plant residues were removed. Subsequently, we dried the soil samples for three days at 50°C. Soil P availability was determined using a Swiss reference method which extracts P with CO_2 saturated water (P_{CO2}, Table 2.2) according to FAL (1996). In the extract P was measured by malachite green colorimetry (Ohno and Zibilske, 1991). The treatments were classified by P availability classes as suggested in Flisch et al. (2009). Total P was extracted from dried samples after incineration (550°C for 8 hours) with 0.5 M H_2SO_4 at room temperature. P was measured by colorimetry as described above. Total N and carbon (C) were analysed on dried and pulverized soil samples using a CNS analyzer (Thermo Finnigan, Milan, Italy).

2.3.5. Plant sampling and analysis

We sampled plant biomass at an early growth stage when plants have a high nutrient demand. The growth stage was determined by stem elongation to emergence of the inflorescence described as stage 2 to 3 in Jeangros et al. (Jeangros et al., 2001) of Orchard grass (*Dactylis glomerata)* which was present at all locations. Sampling generally took place 1 to 4 days prior to regular harvests. Except the first sampling in V and W which was a few weeks before the first regular harvest to get plant material at the appropriate growth stage. For the sampling a randomly selected area of 50 cm x 50 cm with at least 50 cm distance to the plot border was cut using electric scissors 4 cm above the ground. Different sampling areas within the plots were used for the individual cuts throughout the season. Immediately after cutting the plant material was separated into the three botanical fractions: grasses, legumes and forbs. We weighed the plant material after drying at 60°C for three days. After drying samples were milled (particle size ~1 mm) using a cutting mill (Retsch Gmbh, Germany). Subsequently, we pulverized a subsample using a ball mill (Retsch GmbH, Germany). For P and K analysis, we incinerated milled samples at 550°C for 8 hours and solubilized the ashes in 15 M nitric acid at room temperature. We measured the nutrient concentrations on dry matter basis for the

three botanical fractions grasses, legumes and forbs. The P and K concentration was measured with an ICP-MS (Agilent, USA). The N concentration was measured by dry combustion with a CNS analyzer (Thermo Finnigan, Milan, Italy).

2.3.6. Calculations

We calculated relative yields as the percentage of the yield indication for the respective management intensity (Table 2.3) as suggested in Flisch et al. (2009). Bulk nutrient concentrations were calculated as weighted means:

$$NC_{bulk} = (NC_{grasses} \times DM_{grasses} + NC_{legumes} \times DM_{legumes} + NC_{forbs} \times DM_{forbs}) / DM_{total} \quad [1]$$

where NC and DM are the nutrient concentrations and the dry matter, respectively. The P concentrations were multiplied with yield for each harvest and fraction separately and summed as total P uptake. P uptake was subtracted from the P fertilizer input to obtain the annual P balance.

We interpreted the concentrations of the grass fraction by using the following critical values, all in mg g^{-1} DM: 2 (Whitehead, 2000) and 2.6 (Bailey et al., 1997a) for P; 20 for N and 20 for K (Bailey et al., 1997a) obtained mostly for perennial ryegrass. Nutrient ratios N:P, N:K and K:P were calculated using the measured concentrations for fractions and the calculated bulk concentrations, respectively. For interpretation for the grass fraction we used the norm ratios established for perennial rye grass in a well balanced fertilizer pot experiment: N:P= 9, N:K= 1.2 and K:P= 8.5 (Bailey et al., 1997a).

For the PNI we first verified the critical curve used for calculation. Therefore, N and P concentrations of the grass samples from all treatments with sufficient K input were correlated (see Figure A.1) resulting in the function: $P = 0.7 \times N + 2.0$ (r^2=0.61, ***, n=130). Neither the slope nor the intercept of the curve were significantly different from the function proposed by Duru and Thélier-Huché (1997). Consequently, we calculated the PNI for each fraction using the formula of Duru and Thélier-Huché (1997):

$$PNI = 100 \times Pm / (0.065 \times Nm + 1.5) \quad [2]$$

where Pm and Nm are the measured P and N concentrations in mg g^{-1} in the fractions. Despite that the critical curve has not been established for legumes and forbs we used the same equation (2) to calculate the bulk sward PNI (PNI_{bulk}). For PNI_{bulk} we used the weighted mean Nm_{bulk} and Pm_{bulk} concentration as calculated in equation 1. The PNI in non-leguminous

plants (PNI_{nonleg}) was calculated with equation 2 using the weighted mean Nm_{nonleg} and Pm_{nonleg} concentrations of grasses and forbs (equation 3).

$$NC_{nonleg} = (NC_{grasses} \times DM_{grasses} + NC_{forbs} \times DM_{forbs}) / DM_{grasses+forbs} \quad [3]$$

The PNI indicates a theoretical turning point between deficient and luxurious nutrient supply at 100. A practically applicable range for sufficient nutrient supply was proposed as 80 to 120 (Duru and Thélier-Huché, 1997). Below 80 the plants suffer P deficiency and above 120 luxurious consumption is indicated.

We calculated the DRIS P index for the grass fraction by the following equation using norm ratios of nutrients established in perennial ryegrass from Bailey et al. (1997a):

$$DRISA = [f(A/B) + f(A/C) - f(D/A) + ... + f(A/M)]/z \quad [4]$$

where $f(A/B) = \frac{A/B}{a/b} - 1\frac{1000}{CV}$ when $A/B > a/b$

or $f(A/B) = 1 - \frac{A/B}{a/b}\frac{1000}{CV}$ when $A/B < a/b$

A to M represents the nutrients used in the calculations (P, N, K, Ca and Mg in this study). Capital letters are the measured nutrient concentrations and small letters reflect the norm ratio for the respective nutrients. The denominator z (here 10) is the number of functions used to calculate DRIS P. The selected norm ratios were P:K, P:Ca, P:Mg, N:P, N:K, N:Ca, N:Mg, K:Ca, K:Mg and Mg:Ca and the respective values and CV were obtained from Bailey et al. (1997a). In theory, negative DRIS P reflects limitation and positive values surplus supply.

2.3.7. Statistical analysis

Statistical analyses were performed with R version 2.8.1 (R-Development-Core-Team, 2008). We analyzed differences of nutrient concentrations between botanical fractions and harvests for individual treatments using a two factorial ANOVA followed by comparison of mean values using the Student-Newman-Keuls test (SNK). A three way ANOVA including treatment as a factor was only applicable for V and the W experiment with the mineral fertilizer treatments. Because experiments of the three locations had different designs, fertilizer treatments were compared between sites with a F-test on pooled data for all harvests. If the F-test was significant at $p<0.001$ a test for least significant difference (LSD) with an alpha of 0.05 was conducted using the library agricolae v. 1.09 (Mendiburu de, 2009). We

always applied the Holm adjustment for p-values for multiple comparisons. Prior to analysis, we transformed percentage data of relative yields to their arc sin. Correlation coefficients (ρ) and their level of significance were calculated by the Pearson product-moment correlation. Significance levels are: ns. = not significant, * = $p<0.05$, ** = $p<0.01$ and *** $p<0.001$. Trend lines were fitted to graphs in SigmaPlot for Windows version 11.0 (Systat Software, 2008) using non-linear regression. We used a polynomial- linear, exponential rise to maximum and Gaussian peak procedure to fit the linear, exponential and the peak trend lines, respectively.

2.4. Results

2.4.1. Dry matter yield

Mean total dry matter yields ranged from 4.3 to 14.8 t ha^{-1} yr^{-1} (Figure 2.1). The yields of N1P1K1 treatments were close to the indicated yields. In general, differences were more pronounced between management intensities and location than between treatments within each location. A greater number of harvests and vegetation days per year (Table 2.1), led to higher total yields in this study. Yields of single harvests declined during the season, regardless of management (data not shown). Mean relative yields to the indicated yields ranged from 59% in W-N0P0K0 to 109% in B-N1P1K2org/min (LSD = 23.3). We observed significant effects of fertilizer treatments within the locations V and W. Treatments fertilized as or higher than recommended were close to the indicated yields. A significant yield reduction was found in treatments, in which one or more nutrients had been omitted, with a reduction in relative yield in V-N1P0K0, W-N1P1K0 and W-N1P0K1 by 16, 22 and 19%, respectively. We observed the most severe reduction by about 40% in W-N0P0K0 that received no fertilizer at all. In B, we observed no differences due to the fertilization. The lower total yield observed in B-N1P1K2org (Figure 2.1) compared to the other three swards in B resulted from the higher altitude of this particular grassland (Table 2.1).

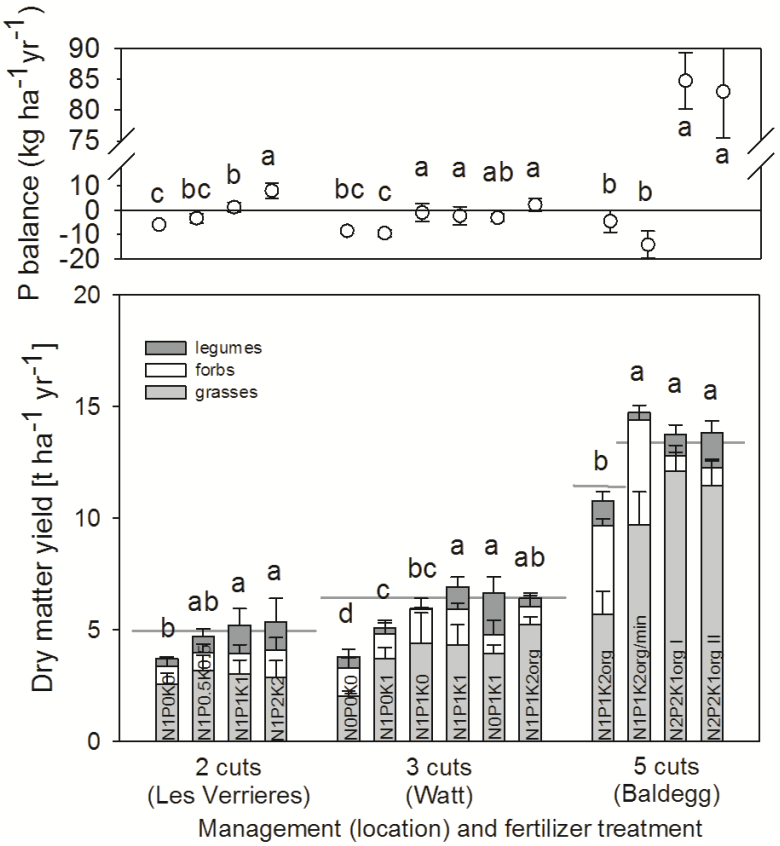

Figure 2.1: Mean annual P balance and dry matter yield for the botanical fractions grasses, legumes and forbs in 2008 as affected by management (location) and fertilizer treatment. Error bars indicate standard deviation. Grey horizontal lines show the indicated yield for each management. Different letters indicate significant differences of the P balance and total annual yield within a location (LSD test at alpha = 0.05, with Holm adjusted p values).

2.4.2. Soil P availability and P balances

In general, the P_{CO2} extracted from soils increased in the order V<W<B (Table 2.2). Within each location, P_{CO2} increased with increasing P fertilizer application. The corresponding P nutrition status classes for P_{CO2} ranged from low to accumulated. In V, all treatments had a low soil P status except V1P2K2 which was adequately P supplied. In W, the P fertilized treatments were classified as adequate whereas in P0 treatments the P availability was low. In B the P nutrition status of B-N1P1K2org/min was classified as high and the other three as P enriched. The estimated annual P balance in 2008 (Figure 2.1) ranged from a deficit of -14 in B-N1P1K2org/min to a surplus of +85 kg ha^{-1} yr^{-1} in B-N2P2K1org I. Except B-N1P1K2org/min, all P1 treatments were not significantly different from zero. In contrast, P treatments without or surplus P fertilization were significantly lower or higher than zero, respectively.

2.4.3. Botanical composition

The botanical fractions grasses, legumes and forbs were composed of different species number and composition (Table 2.1). The fractionation of aboveground biomass on a dry weight basis is shown in Figure 2.1. Grasses presented the main fraction in all grasslands and fertilizer treatments. The highest grass proportions were observed in B-N2P2K1org I+II (78 and 88%) and W-N1P1K2org (72%). The proportion of grasses fluctuated over the season, with the grass fraction dropping below 50% in ten out of 46 harvests but only in two cases below 40% (data not shown). Losses in the grass fraction were compensated by gains in legumes or forbs or both, depending on the fertilizer treatment. The maximum proportions of legumes were found in the fertilizer treatments V-N1P1K1, V-N1P2K2 and W-N0P1K1 with balanced or high P and K application. In B-N1P1K2org/min, a generally higher fertilizer N application fostered forbs up to 56% while the legume fraction remained constant between 1 to 5 % at all harvests. Low legume proportions occurred in B-N2P2K1org II, W-N1P0K1 and W-N1P1K0. The lowest proportions of forbs were found in W-N0P1K1, W-N1P1K2org and in B-N2P2K1org I+II.

2.4.4. Nutrient concentrations in botanical fractions

Fertilization with P, N or K increased the concentrations of the particular nutrient in the aboveground plant biomass of all botanical fractions (Table 2.4). In general, N concentrations were less variable over time and fraction than those of P and K concentrations which differed significantly between fractions ($p<0.0001$). Different fertilizer effects on P concentration in the botanical fractions were indicated by a significant interaction ($p<0.0001$) between treatment and botanical fraction. When P supply was omitted (W-N0P0K0, W-N1P0K1 and V-N1P0K0), forbs and legumes showed significantly higher P concentrations than grasses (non-significant trend for forbs in V-N1P0K0). Under sufficient P supply, P concentrations tended to be lower in legumes than in grasses and forbs. Forbs in the treatments B-N1P1K2org and B-N1P1K2org/min had significantly higher P concentrations than grasses and legumes. As expected, the N concentrations in legumes were always higher than in grasses and forbs. The N concentrations of grasses and forbs were usually similar. The K concentrations were generally highest in the grass fraction (except in V-N1P0K0, B-N1P1K2org and B-N2P2K1org I) and lowest in legumes.

The coefficient of variation of P and K concentration within each treatment was lower in grasses than in legumes and forbs (Table 2.4). The coefficient of variation of P concentration

for the total dataset was similar in grasses and forbs and lowest in legumes, with coefficients of variance of 30, 35 and 21, respectively.

The above described differences in P, N and K concentration in the botanical fraction were reflected in N:P and K:P ratios (data not shown). The N:P were significantly higher in legumes than in grasses and forbs, with ranges of mean N:P from 11 to 17, 4 to 11 and 5 to 10, respectively. Although not always significant forbs were mostly located between grasses and legumes. For K:P the differences were less pronounced but K:P tended to be lower in legumes than in grasses and forbs where the ratio was intermediate. The observed ranges of mean K:P in the treatments were 4 to12, 4 to 10 and 2 to 8 for grasses, legumes and forbs, respectively.

The significant change in PNI caused by legumes and forbs is shown in Figure 2.2. The presence of legumes changed the PNI_{bulk} of a value equivalent to about half of the legume proportion on yield. For example, a legume fraction of 40% of the sward dry matter lowered the PNI by 20. The difference between PNI in the non-leguminous fraction and PNI in grasses reflected a higher variation of PNI_{nonleg} compared to $PNI_{grasses}$ caused by the presence of forbs. The changes of PNI_{nonleg} ranged from -8 to 35 (ideally close to zero). Additional we observed a significant trend of the forb fraction to increase PNI_{nonleg}. For DRIS P (data not shown) the variation within fractions of a treatment was higher than for PNI. DRIS P in legumes tended to be higher than in grasses. Forbs were again intermediate.

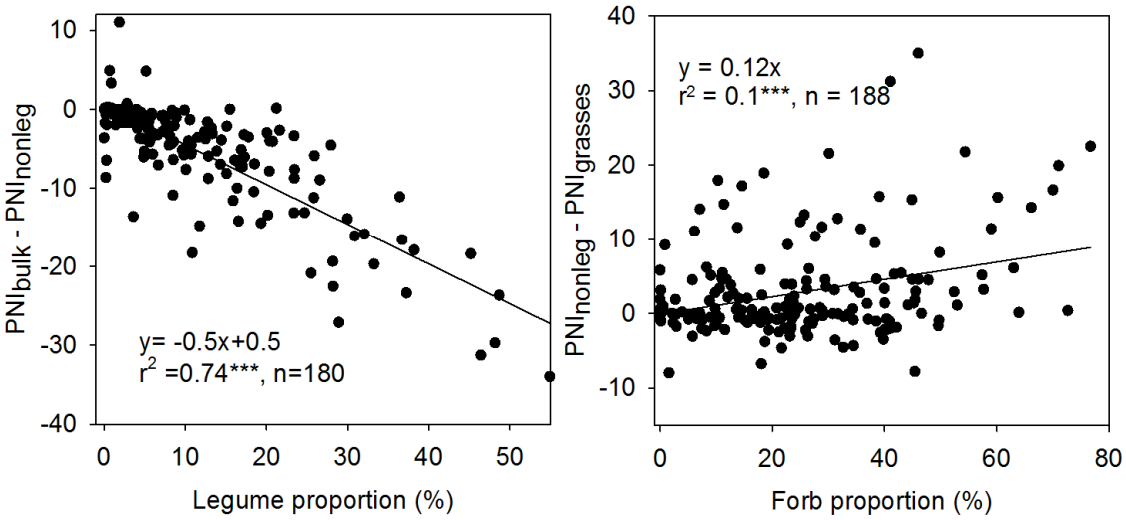

Figure 2.2: Left: Effect of legume proportion on the PNI in bulk biomass (PNI_{bulk}) shown as difference between PNI_{bulk} and PNI in non-leguminous plants (PNI_{nonleg}). Right: Effect of forb proportion on the PNI in non-leguminous samples, shown as the difference between PNI_{nonleg} and PNI in grasses ($PNI_{grasses}$). The Pearson correlation coefficient (ρ) in legumes and in forbs is shown for all samples Significance level *** indicates $p<0.001$.

Table 2.4 Mean annual N, P and K concentrations (g kg^{-1}) and coefficients of variation (CV) in the aboveground biomass of the botanical fractions grasses (G), legumes (L) and forbs (F). The total number of replicates (n) consists of four spatial replicates in the field (except N1P1K1org/min: five replicates) and two, three and five cuts in 2008 in Les Verrières, Watt and Baldegg, respectively. Absence of legumes or forbs reduced the number of replicates in some cases. Different letters indicate significant differences between the botanical fractions within a treatment according to a SNK test at p<0.01.

Location	Treatment	Botanical	Nitrogen				Phosphorus				Potassium		
			n	mean	p<0.01	CV	n	mean	p<0.01	CV	mean	p<0.01	CV
Les Verrières	N1P0K0	G	8	19.4	b	8	8	1.7	b	20	12.0	a	14
		L	8	36.2	a	8	8	2.4	a	53	7.9	b	52
		F	8	19.2	b	12	8	1.8	b	52	9.9	ab	56
	N1P0.5K0.5	G	8	19.9	b	13	8	2.6	a	9	15.7	a	10
		L	8	35.1	a	1	8	2.7	a	51	9.4	b	51
		F	8	19.3	b	14	8	2.8	a	52	14.7	a	52
	N1P1K1	G	8	20.7	b	13	8	3.3	a	12	19.0	a	16
		L	8	37.0	a	1	8	3.1	a	52	11.9	c	53
		F	8	19.6	b	13	8	3.2	a	51	16.1	b	53
	N1P2K2	G	8	19.6	b	12	8	3.5	a	14	22.8	ab	7
		L	8	35.9	a	1	8	3.2	a	52	20.7	b	54
		F	8	19.3	b	15	8	3.4	a	52	24.7	a	54
Watt	N0P0K0	G	12	17.7	b	17	12	1.7	c	20	12.9	a	17
		L	12	29.5	a	18	12	2.3	a	51	8.8	b	50
		F	12	17.9	b	2	12	2.0	b	45	10.5	b	44
	N1P0K1	G	12	20.1	b	16	12	1.7	b	23	21.3	a	16
		L	10	37.3	a	8	11	1.9	a	44	12.7	b	52
		F	12	19.5	b	4	12	2.1	a	46	18.2	a	47
	N1P1K0	G	12	20.7	b	7	12	3.1	a	19	13.0	a	22
		L	5	33.3	a	14	12	3.5	a	47	7.5	b	57
		F	12	20.8	b	13	12	3.4	a	44	11.9	a	50
	N1P1K1	G	12	19.6	b	13	12	3.2	a	26	21.2	a	24
		L	12	31.3	a	7	12	2.9	b	46	11.8	c	64
		F	12	20.4	b	3	12	3.2	a	45	16.0	b	50
	N0P1K1	G	12	20.0	b	5	12	3.4	a	23	20.9	a	22
		L	12	28.7	a	21	12	2.8	b	46	12.2	c	59
		F	12	19.9	b	2	12	3.3	a	44	16.6	b	49
	N1P1K2org	G	12	21.2	b	3	12	2.4	a	26	25.0	a	20
		L	12	32.5	a	18	12	2.5	a	47	15.6	c	51
		F	12	21.0	b	9	12	2.5	a	45	20.4	b	45
Baldegg	N1P1K2org	G	20	25.1	b	29	20	4.1	b	14	30.9	a	12
		L	19	42.1	a	12	19	3.9	b	18	24.8	b	12
		F	20	25.2	b	26	20	5.0	a	24	35.0	a	15
	N1P1K2org/min	G	25	28.1	c	17	25	3.9	b	15	29.9	ab	9
		L	18	37.9	a	17	19	3.7	b	21	25.4	b	7
		F	25	30.5	b	19	25	4.4	a	11	31.5	a	10
	N2P2K1org I	G	20	29.0	b	31	20	4.5	a	17	29.8	ab	28
		L	16	39.2	a	20	16	3.4	b	12	28.4	b	17
		F	13	32.4	b	23	13	4.8	a	5	37.3	a	21
	N2P2K1org II	G	20	28.7	b	18	20	4.3	a	12	29.2	a	17
		L	13	34.5	a	19	13	3.1	b	13	20.6	b	41
		F	15	30.1	b	14	15	4.9	a	16	28.8	a	26

2.4.5. Nutrient indicators in grasses

Phosphorus concentrations in grasses were significantly affected by locations and treatments (Table 2.4). Generally, B had higher P concentrations than V and W. Concentrations of the treatments V-N1P0K0, W-N0P0K0 and W-N1P0K1 were below the critical value of 2 mg g^{-1}. W-N1P1K2org was the only treatment fertilized as recommended to fall below 2.6 mg g^{-1}. In contrast, the oversupply with P in V-N1P2K2 and B-N2P2K1org I+II did not result in significantly higher concentrations than in the treatments fertilized with P as recommended. The mean annual N concentrations in grasses were generally higher in B than in V and W (Table 2.4), but we observed no treatment differences within a location. Only W-N0P0K0 and V-N1P2K2 had mean N concentrations below the critical value of 20 mg g^{-1}. Similar to P and N, K concentrations were lower in V and W than in B (Table 2.4). In V and W, K concentrations were significantly higher in K fertilized than in unfertilized treatments where K concentrations were lower than 20 mg g^{-1}. Concentrations of K in the K-overfertilized treatments (V-N1P2K2, B-N1P1K2org and B-N1P1K2org/min) were not significantly higher than in the treatments where K was applied as recommended (V- and W-N1P1K1 and B-N2P2K1org I+II).

The N:P ratios in grasses ranged from 6 to 12 (Figure 2.3). Mean ratios were higher than 9 in all non-P-fertilized treatments. They were lower in all treatments where P was applied except W-N1P1K2org where it was about 9. The K:P ratios ranged from 4 to 12, with minimum and maximum in W-N1P1K0 and W-N1P0K1, respectively, where K or P were omitted. We found no significant difference of K:P ratios between treatments with both P and K fertilized as recommended, and treatments with surplus fertilization of P or K or both. The norms for N:P ratio (9) and K:P ratio (8.8) used for the calculation of the DRIS indices were higher than the mean ratios of all grass samples (7.5 and 7.3, respectively) observed in our study.

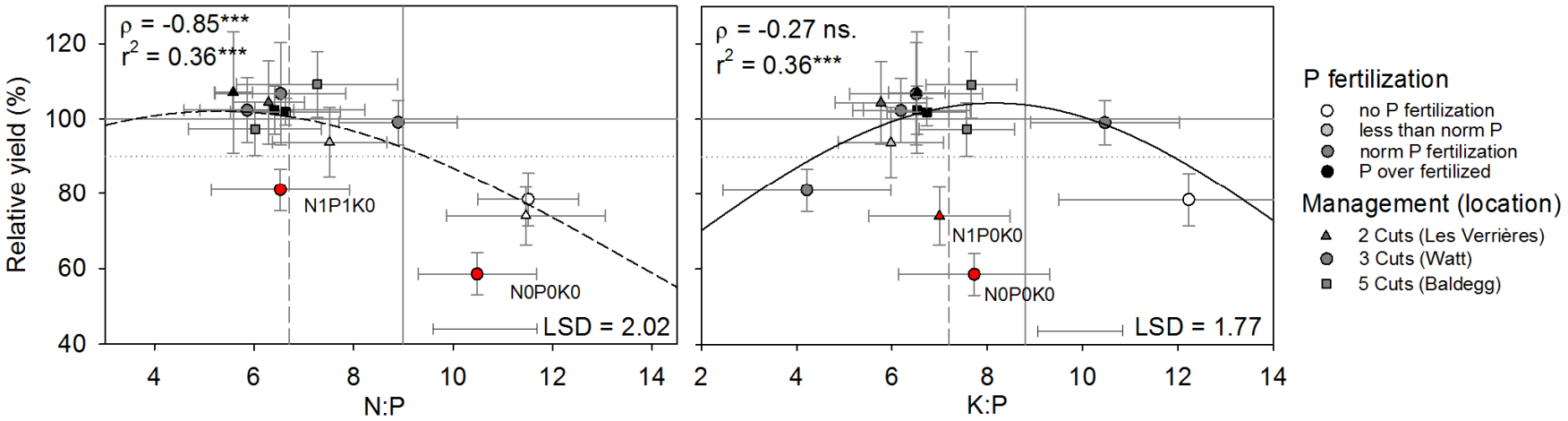

Figure 2.3 Figure 3: Mean annual K:P and N:P ratios in aboveground biomass of grasses sampled in vegetative growth stage as affected by P fertilization (color) and management (location=symbol) and their relationship to relative annual yield. Vertical grey lines represent the critical ratios (solid) and mean K:P and N:P ratios in our study (dashed), respectively. Horizontal lines represent 100 (solid) and 90 % (dotted) of relative yield, respectively. Error bars indicate standard deviation. Treatment differences are shown as least significant differences according to a LSD test with Holm adjusted p values (alpha = 0.05). For the relationship to relative yield the Pearson correlation coefficient (ρ) is shown. Trend lines were fitted as Gaussian peak (3 parameter, $y=a\times e^{(-0.5\times((x-x0)/b)2)}$) excluding the labeled treatments (red symbols). Significance levels are: ns. = not significant, * = p<0.05, ** = p<0.01 and *** = p<0.001.

The mean PNI in grasses ranged from 62 in V-N1P0K0 to 137 in B-N2P2K1org I (Figure 2.4). The PNI of non-P-fertilized treatments were less than 80 and were significantly lower than for treatments with P application. Fertilization at the recommended rate resulted in a mean PNI between 80 and 120, with the exception of B-N1P1K2org which had a PNI of 132. The mean PNI values above 120 in V-N1P2K2 and B-N2P2K1org I+II reflected surplus supply, but the values were not significantly different from those in the treatments with P fertilization as recommended.
Mean DRIS P ranged from 9 in W-N1P0K1 to 31 in B-N2P2K1org I (Figure 2.4), being significantly lower in non-P-fertilized treatments and W-N1P1K2org than in the P fertilized treatments. Treatments fertilized above recommendation were not different from treatments that were P fertilized as recommended.

2.4.6. Plant indicators in relation to relative yield

The nutrient ratios reflected a peak shaped relationship to relative yield as suggested in Walworth and Sumner (1987). A N:P ratio higher than 9 was related to yield reduction as reflected by the treatments V-N1P0K0, W-N0P0K0 and W-N1P0K1 (Figure 2.3), indicating P limitation. For K:P ratios we found yield limiting treatments over the whole range from 4 to 12. However, above 10 and below 5 only the treatments without P and K fertilization were located, indicating P and K limitation, respectively. The treatments with reduced yield which lay between 5 and 10 were neither P nor K fertilized, indicating a co-limitation.
The relationship of P concentrations, PNI and DRIS P of grasses to relative yield showed a “Mitscherlich” (Mitscherlich, 1928) -type of function (Figure 2.4) with strong increases at low indicators and no change of relative yield at high P indicators. We found no significant yield increase above 2.1 mg P g^{-1}, for a PNI equal to or higher than 80 and for positive DRIS P values. The P concentration, PNI and the DRIS-P tended to be lower in V-N1P0.5K0.5 and W-N1P1K2org than in all other P1-fertilized treatments but did not fall below the critical values mentioned above.
The correlation coefficient between relative yield and N:P ratio, PNI or DRIS P were higher (ρ >0.80) than between relative yield and P concentration (ρ=0.64).

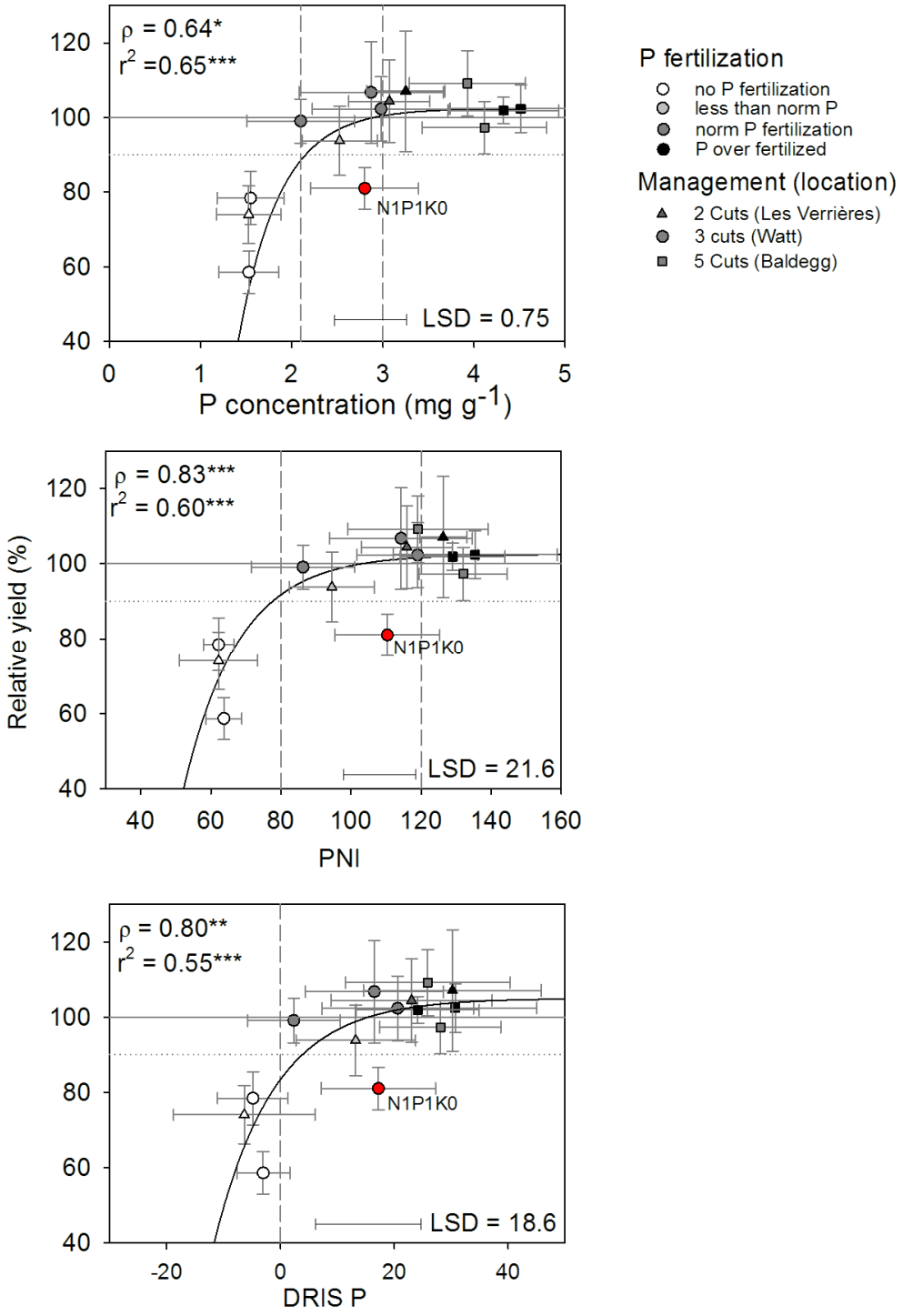

Figure 2.4: Mean annual P concentration (mg g^{-1}), PNI and DRIS P in aboveground biomass of grasses sampled in vegetative growth stage as affected by P fertilization (color) and management (location=symbol) and their relationship to relative annual yield. PNI and DRIS P were calculated as proposed by Duru and Thélier-Huché (1997) and Bailey et al. (1997a), respectively. Vertical grey lines (dashed) represent the critical P concentrations of 2.1 and 3 mg g^{-1}, the DRIS P of zero and PNI of 80 and 120, respectively. Horizontal lines represent 100 (solid) and 90 % (dotted) of relative yield, respectively. Error bars indicate standard deviation. Treatment differences are shown as least significant differences according to a LSD test with Holm adjusted p values (alpha = 0.05). For the relationship to relative yield the Pearson correlation coefficient (ρ) is shown. Trend lines were fitted as exponential rise to maximum (3 parameter, $y=y0+a\times(1-e^{(-b\times x)})$) excluding the labeled treatments (red circles). Significance levels are: ns. = not significant, * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

2.5. Discussion

2.5.1. Yield and soil phosphorus availability reflect long term P fertilization

The dry matter yields in treatments fertilized according to or higher than recommended (Figure 2.1) agreed with the indicated yield of the respective management and altitude in Switzerland (Flisch et al., 2009). Likewise, relative yields to the indicated yield were higher than 90% and the treatments within the range of ± 10% of the indicated yield were not significantly different. Relative yields were less than 90% in treatments where P, K or both nutrients were omitted and illustrate the yield reduction due to insufficient P and K supply.

The P_{CO2} reflected the long term fertilization regime and the above described yield response. The even P balance in treatments fertilized according to recommendation (P1) indicated a sufficient P nutrition status in 2008. Thus, we consider the P1 treatments as sufficiently P supplied for plant biomass production, whereas P0 and P2 treatments represent a P deficient and surplus status, respectively.

2.5.2. Phosphorus fertilization in relation to N and K affects botanical composition

The botanical composition of each grassland used in this study was typical for agricultural permanent grasslands of the respective management intensity and local climate conditions (Dietl, 1995; Klötzli et al., 2010). Grasses were the main fraction on all locations. Grass fractions were highest in treatments with high N fertilization, either in relation to P and K or with high NPK fertilization (most pronounced in B-N2P2K1 I+II). This agrees with Ledgard et al. (1996) who reported that increased N supply mostly fostered grasses and Høgh-Jensen and Schjoerring (2010) who reported an increase in grasses and decrease in legumes when P and K were applied additional to high N. Inversely, the P and K fertilization without N decreased the grass and increased the legume fraction. This was also observed by Thöni (1964) in a grassland of similar management and botanical composition as W. The absence of P and/or K fertilization reduced the legume fraction. This effect was more pronounced for W-N1P1K0 confirming the finding of Bailey and Laidlaw (1998) that white clover was more curtailed by moderate deficiency of K than of P. For forbs, our findings were not

consistent between treatments and locations. Literature about fertilizer effects on forbs is scarce and largely concerns single species (Whitehead, 2000).

2.5.3. Botanical composition affects interpretation of sward P nutrition status

Changes in mineral nutrient concentrations during growth are well documented for European agricultural grassland (Daccord et al., 2001; Thöni, 1964; Whitehead, 2000). By sampling at a young vegetative stage of grasses we limited differences in nutrient concentration induced by growth stage. However, legumes and forbs may have been at different growth stages at the different harvests. This may be one reason why the variation of P concentration in the legumes and forbs fractions was higher than in the grass fraction.

Nevertheless, the differences of P, N and K concentration between harvests (data not shown) were analogous in the three botanical fractions. Therefore, they could largely be ascribed to the timing of fertilizer application and seasonal differences of nutrient availability. We observed substantial differences of P, N and K concentrations between botanical fractions, as previously reported by e.g. Whitehead (2000). That we found higher P concentrations in legumes than in grasses under P limiting conditions agrees with Gallet et al. (2003b). They observed that white clover grown alone had higher P concentration than perennial ryegrass grown alone in the zero P fertilizer treatment of a pot experiment carried out with three different soils. In contrast, Mackay et al. (1995) found no difference in P concentration between legumes and bulk pasture samples over a wide range of plant P concentrations. However, nearly all of their samples had concentrations higher than 2 mg P g^{-1} which means that the availability of P was not limited. Additionally, Mackay et al. (1995) did not further separate their bulk samples into grasses and forbs. A better acquisition of P and K by legumes in mixed stands with grasses under N deficiency than under high N supply was shown by Jouany et al. (2005) for P and by Øgaard and Hansen. (2010) for K. We confirmed this observation for P without severe N deficiency.

Jouany et al. (2005) found that diagnosis of P nutrition status with the PNI in bulk sward samples underestimated the P nutrition status when a high proportion of white clover was present in the sward. That is caused by the higher N concentrations in

legumes. They proposed to base the diagnosis on the non-leguminous fraction to avoid surplus P fertilizer recommendation. As another possibility they suggested to correct the PNI for the proportion of legumes in the sward (Jouany et al., 2005). We observed a similar negative relationship between the total legume proportion (mixed species) and the PNI in our swards (Figure 2.2). It shows that a correction of the bulk sward PNI by adding half of the total legume proportion ($PNI_{corrected} = PNI_{bulk} + 0.5 \times$ %legumes) is appropriate. Similarly, N:P ratios in bulk samples underestimated the P nutrition status in presence of legumes (data not shown). Additionally, we observed a highly variable and generally positive effect of forbs on PNI when the non-leguminous fraction was used (Figure 2.2). Although relatively small, this effect was significant and could lead to an overestimation of P nutrition status when high proportions of forbs are present. Such an overestimation could be critical when the P nutrition status is close to deficiency or or is deficient.

Since grasses are the most important yield component and reflect the P availability reliably in grasslands, we recommend the analysis of the grass fraction sampled at a defined and young vegetative stage.

2.5.4. Interpretation of the P indicators in the grass fraction

Critical P concentrations

The P concentration of 2.1 mg g^{-1} in grasses above which we found no significant yield increase falls into the range between 2 and 6 mg P g^{-1} proposed by Whitehead (2000) as sufficient for perennial ryegrass. However, it is below the average range of 2.4 to 4.1 mg g^{-1} of the Swiss fertilizer recommendation and is lower than the critical concentration of 2.6 mg g^{-1} suggested by Bailey et al. (1997b). However, the range of ± 10% of indicated yield that we considered as satisfactory might not be appropriate to all grassland management purposes. If 10% yield decreases cannot be accepted a critical value of 2.4 or 3 mg P g^{-1} corresponding to 95 and 100% of relative yield, respectively, could be used. Further, the interpretation of nutrient concentrations in relation to forage quality can be different. For example, roughage should have a minimum P concentration of 3 mg g^{-1} to cover dairy animals requirement (Arrigo, 1999). In our study, we restricted the interpretation of P to its function as an element potentially limiting grassland biomass production. However, P concentrations of 3 mg g^{-1} were

reached by recommended P fertilization and the maximum of the response curve was observed at a P concentration of 3.5 mg g^{-1}. Phosphorus concentrations higher than 3 mg g^{-1} (Figure 2.4) did not cause a yield increase. Such luxurious consumption indicates inefficient use of fertilizer P. If P application exceeds plant P uptake, P accumulates in the soil and P losses increase (Watson et al., 2007).

Nutrient ratios

We found optimal ranges from 6 to 8 for N:P and from 6 to 9 for K:P. The N:P ratios of non-P-fertilized treatments were above 10 (Figure 2.3). This agrees with the critical N:P ratio of 10 above which a P limitation can be indicated in terrestrial plants (Güsewell, 2004), but is lower than the critical ratio of 14.5 reported by Venterink et al. (2003) for wet- and grasslands managed at low intensity. The large variance of critical N:P ratios in literature might be partly explained by the variation between species (Fujita et al., 2010; Koerselman and Meuleman, 1996) and growth stages. In agricultural grassland, plants were usually sampled at a defined stage (Dampney, 1992; Whitehead, 2000) keeping such variation low. Greenwood et al. (2008) related different N:P in wild and cropped plants to differences in the tissue composition. They hypothesized that lower N:P in crops than in wild species is related to a lower proportion of storage tissue compared to growth related tissue. Such a difference could explain the higher N:P found by Venterink et al. (2003).

The norm N:P ratio of 9 (Bailey et al., 1997a) could also be interpreted as a critical value here. It divided all P fertilized from the unfertilized treatments which were above and below 90% relative yield, respectively. No yield relevant N deficiency could be detected above N:P of 5.5. That is supported by the indicated peak shaped trend line in Figure 2.3 with a relatively flat top between 5.5 and 10. We speculate that including treatments with severe N deficiency would change the slope of the curve and thus result in precise critical ratios. But the general form of the curve would still be valid. However, co-limitation of P and N might be present in the unfertilized treatment W-N0P0K0. That P-limitation was indicated instead of N-limitation in W-N0P0K0 might be due to N inputs others than by fertilizer as suggested by Fujita et al. (2010). They reported a shift to P-limitation caused by N deposition for eight different grassland species. The fixation of N_2 by legumes which grew on all investigated plots would probably foster such an effect. Still, the mean N concentration in W-N0P0K0 was the

lowest measured in this study, but the growth limitation by N was probably less severe than by P.

The K:P ratios indicated no P-limitation in W-N0P0K0 and V-N1P0K0 because the co-limitation by K resulted in ratios similar to sufficient and over fertilized treatments. Nevertheless, P and K concentrations were below 2 mg g^{-1} and 13 mg g^{-1} indicating P and K deficiency, respectively. The exclusively K limited treatment W-N1P1K0 had a K:P of 4 (Figure 2.3) suggesting that in agricultural grassland the critical K:P ratio may be higher than the 3.4 suggested by Venterink et al (2003). Between 5 and 10, treatments were not or co-limited by P and K. Thus, K:P above 5 and below 10 cannot be used to identify a P deficiency. The peak shape of the fitted curve (Figure 2.3) reflects the yield decreases exclusively caused by P or K deficiency. The norm ratio of 8.8 (Bailey et al., 1997a) was higher than the mean K:P (7.3) and the maximum of K:P (8.1) of the fitted trend line (Figure 2.3) in our study. The norm ratio of 8.8 separated the non-P-fertilized treatment W-N1P0K1 from the rest, which allows its interpretation as a critical ratio here as well. However, these findings indicate that the interpretation of nutrient ratios requires two critical ratios for each nutrient framing a norm.

Phosphorus nutrition index

The critical curve for the PNI from Duru and Thélier-Huché (1997) proved applicable to the grass samples in our study. The fitted exponential trend line confirmed the applicability of the interpretation range of 80 to 120 suggested by Duru and Thélier-Huché (1997). The PNI of 80 corresponded to relative yields of 90 considered satisfactory in our study whereas PNI of 120 and more reflected relative yields of 102 in the horizontal part of the trend line. PNI classified according to P inputs (Figure 2.4) correctly expressed the expected P nutrition status except for B-N1P1K2org where a luxurious P nutrition status was indicated. Possibly, we underestimated the P input by slurry in the B-N1P1K2org treatment because some pigs were also raised on that farm. Pig slurry contains more P than dairy slurry (Flisch et al., 2009) and both slurry types were mixed in the storage tank. This assumption is supported by the high P_{CO2} in the soil. Additionally, the higher yield and N application in B-N1P1K2org/min diluted the P concentration resulting in comparatively lower PNI in N1P1K2org/min than in B-N1P1K2org.

DRIS indices

Despite the similar shapes of the fitted curves DRIS P differentiated less clearly than PNI between treatments with luxurious and recommended P fertilization. The integration of N, K, Ca and Mg in addition to P for the calculation of the DRIS P might result in higher variation for each treatment. Discrepancies within the norm ratios as found in N:P and K:P might also create additional variation. The lower precision of DRIS P than PNI was also reflected by the higher range of P concentrations related to a DRIS P of zero (1.5 to 2.6 mg g^{-1}) than to a PNI of 80 (2 to 2.3 mg g^{-1}). The trend of reduced yield in V-N1P0.5K0.5 and W-N1P1K2org was not linked exclusively to P limitation because PNI and DRIS-P were above 80 and positive, respectively. The yield limitation in V-N1P0.5K0.5 can largely be related to K deficiency because the K concentration was below 18 mg g^{-1} and DRIS K indicated K deficiency. The DRIS P in W-N1P1K2org was close to zero indicating an optimum P nutrition. However, W-N1P1K2org had higher DRIS N and K than P indices (see Annex: Table A.3), suggesting that in spite of positive DRIS P, P was the most limiting nutrient here. Further, in W-N1P1K2org all P indicators showed the worst P nutrition among the P fertilized treatments. This further illustrated the complexity of interpretation of the P indicators under nutrient co-limitation.

2.6. Conclusion

The botanical composition of the swards was influenced by the relation of P (and K) to N input, with higher N input increasing the grass fraction. Input of P fertilizer increased the P concentration in all three fractions but legumes and forbs maintained higher P concentration under P limiting conditions. Grasses were the main proportion of total yield and showed a higher precision for P nutrition status indication when used solitary. We therefore recommend to analyse the grass fraction to evaluate the P nutrition status of permanent grasslands. To improve the accuracy of this evaluation, grasses should be sampled at a defined vegetative growth stage. All P indicators, except K:P when K was deficient, allowed to distinguish between P deficient and P fertilized treatments. Above P concentrations of 2.1 to 2.3 mg g^{-1}, the grasslands produced at least 90% of the indicated yields and yields were not significantly different. Above 3 mg P g^{-1} no yield increase was observed. Thus, this range could be interpreted as sufficient for plant growth and optimal for efficient P fertilizer use in permanent grasslands. For nutrient ratios, we propose the use of optimal ranges instead of one critical ratio to reflect the

sufficient P nutrition status of the sward. We propose ranges from 6 to 9 for K:P and 6 to 8 for N:P. However, the three P indicators integrating N or more nutrients (N:P, PNI and DRIS P) correlated better to relative yield. Among these P indicators, the PNI resulted in the best treatment differentiation allowing to distinguish between deficient, sufficient and surplus P fertilization. The K:P only showed distinct P or K limitation while it failed in case of co-limitation. Only DRIS or a combined use of several nutrient indicators reflected the co-limitation (here with K) correctly. To identify co-limitation correctly we recommend the use of a set of nutrient indicators.

Chapter 3: Derivation of thresholds for three methods estimating plant available phosphorus in the soil

3.1. Abstract

Clear definitions of deficient, sufficient and surplus phosphorus (P) availability are a prerequisite to precisely characterize plant P nutrition status. In agricultural permanent grasslands such thresholds are not yet well established. In this study we aimed to establish thresholds for soil P availability using yield and plant P nutrition status response curves. To estimate soil P availability we used three soil P indicators based on different principles: an extraction method (P_{ox}), an isotopic exchange kinetic (E_{1min}) and an ion sink method (P_{res}). These three soil P indicators were investigated in permanent grasslands managed at different harvest intensities with no (P0), sufficient (P1) or surplus P (P2) inputs since 5 to 20 years. In the 0 to 5 cm soil layer, the P0, P1 and P2 treatments were significantly differentiated by the three soil P indicators. In 20 to 40 cm depth only surplus P fertilized treatments were significantly higher for the three soil P indicators. Correlations of P_{ox}, E_{1min} and P_{res} with yield and P uptake were specific for each location. Thus, the yield response was determined as the percentage of the expected yield. Sward P nutrition status was determined with P concentration and the P nutrition index (PNI) in grasses. For these plant indicators, correlations to P_{ox}, E_{1min} and P_{res} were stronger using all sites combined than for specific locations. For these relationships we established exponential functions with a maximum. In a previous study we identified three thresholds (low, middle and high) defining plant P nutrition status for relative yield (90, 95 and 100%), grass P concentrations (2.1, 3.0 and 3.5 mg P g^{-1}) and the PNI (80, 100 and 120). These thresholds were applied here to define the soil P availability classes deficient, sufficient and surplus for plant growth. Using a 95% confidence range resulted in low, middle and high thresholds of 290, 375 and 510 for P_{ox}, of 5, 7.5 and 14 for E_{1min}, and of 4.5, 7.3 and 14 mg P kg^{-1} soil for P_{res}. Among the three P indicators P_{res} had the strongest coefficients of determination with grass P concentration and PNI, thus it reflected sward P nutrition status better than P_{ox} or E_{1min}.

3.2. Introduction

In agricultural soils of Europe, phosphorus (P) is still accumulating, particularly under grassland in areas supporting high life stock density (Schröder et al., 2010). Environmental impacts such as decline of biodiversity (Wassen et al., 2005) and eutrophication of surface waters have been linked to increased P losses from P-rich grassland (Haygarth, 2005; Watson et al., 2007). Since grasslands cover a large proportion of the agricultural area in Europe, an improved P fertilizer use in grasslands may have a large impact on overall P use efficiency in agriculture. However, grasslands have a high plant biodiversity and may differ strongly in botanical composition, management, soil and environmental conditions. Different sward management and climate are likely to affect short and long-term P availability and uptake and use of P by plants. Thus, widely applicable and reliable indicators for the P nutrition status of grasslands are needed.

The prediction of P availability and uptake by means of soil analysis is particularly difficult in grasslands (Herlihy et al., 2006). A wide range of methods is used today to identify soil P status and to investigate processes affecting P availability in grasslands. For fertilizer recommendations, the soil P status is mostly determined by chemical extractions as routine tests. In principle these can be divided into weak extracts (e.g. H_2O) estimating P in the soil solution (intensity factor) and stronger extracts (e.g. $NaHCO_3$) partly including the soil P reserves (quantity factor). Such routine soil tests have been calibrated to soil P availability mostly based on relationships to yield response or P uptake for different soils and crops. But such relationships are variable (Morel et al., 2000). Chemical soil tests often extract variable P fractions which are not well defined for their role in plant P nutrition (Demaria et al., 2005) or neglect plant availability after mineralization of organic P forms in the soil (Steffens et al., 2010).

Two research approaches frequently used to study P availability and losses to the environment are the isotopic exchange kinetics (IEK) and the degree of P saturation (DPS) which is based on an oxalate extraction of P. The IEK is a well-established tool to estimate the ability of the soil to replenish P in the soil solution in the short and long term. It reflects transfer from soil to solution better than many chemical extractions because it considers both intensity and quantity factors (Morel et al., 2000). A typical parameter for P availability obtained by the IEK is P

exchangeable in 1 minute (E_{1min}). A preliminary threshold for P exchangeable in one minute (E_{1min}) below which yield of field crops declines was found at 5 mg kg^{-1} (Gallet et al., 2003a). Morel et al. (1992) reported similar values of 3.8 and 6 mg P kg^{-1} for 95 and 98.5% of relative yield of a wheat crop. These values have not yet been confirmed for grassland and give no indication for surplus P nutrition of plants. However, Gallet et al. (2003) found maximum P concentration and uptake in grassland at E_{1min} of 18 mg kg^{-1}. That value could be used to indicate surplus P nutrition.

The oxalate extraction of P (P_{ox}) was developed to estimate the risk of P losses by leaching from soils (van der Zee et al., 1987). For that purpose it was related to iron and aluminum oxides which reflect the P buffer capacity of the soil (Schoumans, 2000) to calculate the degree of P saturation (DPS). In addition, it has been used to estimate P availability (Fransson, 2001). However, P availability was calibrated in relation to standard soil P analysis and not to plant P nutrition status. In a pot experiment, Koopmans et al. (2004) observed P deficiency in *Lolium perenne* when P_{ox} was below 640 mg P kg^{-1} soil. In a field study, Bell et al. (2005b) found herbage P nutrition status to be better correlated to DPS than to P_{ox}. However, they identified no plant P deficiency over a wide range of P_{ox}.

The extraction of P with anion resin membranes (P_{res}) is a sink method which simulates the P uptake by roots in the soil. It has been reported to be better correlated to plant P uptake than many chemical soil extractions and to be less affected by soil properties such as pH (Mallarino and Atia, 2005; Qian et al., 1992). Usually, P_{res} is determined on air-dried soil samples. However, if it is determined in connection with determination of microbial P (Kouno et al., 1995) then moist soils are analyzed. Although P_{res} has been used to describe the P nutrition status of grassland (Chen et al., 2000) and although P_{res} is a standard method in Brazil (Cantarella et al., 1998), an interpretation scheme reflecting deficient, sufficient and surplus P nutrition of grasslands has not yet been established.

Although chemical soil extractions are used as routine soil tests they do not always reflect the P availability reliably. The methods introduced above potentially improve estimation of P availability, but the literature relating these methods to plant P nutrition status in the field is scarce. The comparability of many studies is constrained because different methods and plant P indicators are used to identify thresholds. Some studies were conducted as pot experiments

(Koopmans et al., 2004; Tran et al., 1988) and thus values cannot reliably be transferred to field situation. In other studies the investigated soil P status in the soils under grassland did not cover the full range from deficient to surplus P (Bell et al., 2005b; Gallet et al., 2003a). Whereas most studies established thresholds to identify deficient P nutrition the luxurious consumption or oversupply was usually not defined. To ascertain, generalize and extend the reliability of these methods more studies on different soils with wide ranges of P supply are needed.

Our objective was to accurately characterize the P nutrition status of permanent grasslands. Therefore, we used three different approaches (chemical extraction, isotopic exchange kinetics and a ion sink approach) to estimate P availability in the soil as related to plant yield and P nutrition status. To this end, we sampled plants and soils from permanent grasslands which were managed at different intensities and received different P inputs over 5-20 years. We established relationships of the three soil P indicators P_{ox}, E_{1min} and P_{res} with total sward yield response, P concentration and the phosphorus nutrition index (PNI) in grasses. To identify deficient, sufficient and surplus plant P nutrition status we used the thresholds derived in chapter one. These thresholds were applied to the soil P indicators using the established functions to propose soil P status classes. A comparison between the three soil P indicators should eventually reveal differences of the approaches to estimate plant available P in the soil.

3.3. Material and methods

3.3.1. Study sites

We selected three grassland types at three different geographic locations reflecting different management (cutting frequency and fertilizer input) and environmental conditions. The locations were north of Les Verrières (V) in the Jura mountains (46°56’18”N, 6°27’38”E, 1140 m above sea level), 2) Watt (W) near Zürich (47°26’44”N, 8°29’32”E, 500 m above sea level) and 3) in the eastern area of the Lake Baldegg (B) watershed (near 47°11’55”N, 8°31’01”E, 560-780 m above sea level). Environmental conditions and botanical composition have been described in detail in chapter one. The locations V and W are long-term fertilizer trials with different rates of N, P and K applications since 1992 and 1996, respectively. They are managed

by the research institutions Agroscope Changins-Wädenswil and Agroscope Reckenholz-Tänikon, respectively. Location B is an agricultural region dominated by intensive livestock production and grassland management for forage production with high application rates of animal manure for several decades. In B we selected four grasslands with two different soil P status that were managed by farmers in the same way since 20 years and in case of N1P1K2org/min since five years. The swards in V, W and B were cut 2, 3 and 5 times, respectively, in 2008 when the grasslands were sampled.

The soils in V and W were Cambisols with a silty loam texture and a sandy loam texture, respectively (FAO, 1998). Both soils were well developed to a depth of 40 cm (no stones or coarse material). The soils in B can be classified as Eutric Cambisols with textures ranging from loamy sand to silty loam (FAO, 1998). These soils were also free of coarse material except N2P2K1org I where we found stones in the soil layer below 30 cm. The pH, total soil nitrogen, carbon and P are shown in Table 2.2. The average cation exchange capacity (± standard deviation) was 28±3, 20±3 and 24±7 $cmol_c\ kg^{-1}$ for V, W and B, respectively.

3.3.2. Experimental set up and fertilizer treatments

For each treatment, four plots of approximately 2 m x 5 m were used. In the fertilizer experiments V and W, the allocation of the plots was given by the original set up of the experiments (Figure 1.2). On farm in B, four plots of the same size were arranged as spatial replicates within the investigated grassland, with at least 5 meters distance between plots. In one case in B (N1P1K2org/min) a 5^{th} plot was installed because of local inhomogeneity of botanical composition observed during botanical investigation. The treatments were referenced with the following nomenclature: N, P and K stand for the three nutrients followed by 0, 0.5, 1 and 2 for no, less, close to equal and more fertilizer applied than average plant P uptake. Average plant P uptake for the different management intensities was taken from the Swiss fertilization guidelines by Flisch et al. (2009). The suffix org and org/min stands for the use of slurry and combined mineral fertilizer and slurry application. The particular rates of fertilization for each site are shown in chapter one.

In V, four treatments with increasing mineral P and K applications and similar N supply were chosen from a randomized split block experiment. Fertilizers were applied as ammonium nitrate (split in two applications after the winter in March and after the first cut in June), superphosphate and potassium (applied in autumn). In W, five mineral fertilizer treatments from a randomized block experiment (Huguenin-Elie et al., 2006) and an organically fertilized treatment from another block experiment located on the same site were chosen (see Figure 1.2). Fertilizers were applied as ammonium nitrate split in three applications: in spring, after the first and after the second cut), superphosphate and potassium (applied in spring) for mineral N, P and K fertilization, respectively. In the organically fertilized treatment dairy slurry was applied after the first cut in spring. In B, two grasslands were fertilized as recommended and two were over-fertilized with P. Dairy or pig slurry produced on the respective farm was broadcasted at the onset of the vegetation in spring and after each harvest. In the year of study (2008), there was no spring application of slurry in B. In N1P1K2org/min no slurry was applied after the second harvest, but 27 kg N ha^{-1} was supplied in form of ammonium nitrate.

3.3.3. Plant sampling and P indicators

Plant biomass of each regrowth was sampled at an early growth stage (defined as beginning of heading of *Dactylis glomerata*). Sampling took place 1 to 4 days prior to regular harvests. Therefore, we selected a random area of 50 cm x 50 cm with at least 50 cm distance to the plot border and cut the vegetation 4 cm above the ground using electric scissors. Different sampling areas within the plots were used for the individual cuts throughout the season. Immediately after cutting the plant material was separated into the three botanical fractions: grasses, legumes and forbs. We weighed the plant material after drying at 60°C for three days. All fractions and harvests were summed as total dry matter yield (Table 1). Relative yields were calculated using the yield expected for the respective management intensity under Swiss conditions reported in Flisch et al. (2009). After drying, the samples were milled (particle size ~1 mm) using a cutting mill (Retsch Gmbh, Germany). Subsequently, we pulverized a subsample using a ball mill (Retsch GmbH, Germany). For P and K analysis, we incinerated milled samples at 550°C for 8 hours and solubilized the ashes in nitric acid (65%) at room temperature. The P and K concentration was measured with an ICP-MS (Agilent, USA). The N

concentration was measured by dry combustion with a CNS analyzer (Thermo Finnigan, Milan, Italy).

We measured the nutrient concentrations on a dry matter basis for the three botanical fractions grasses, legumes and forbs. Total P uptake was calculated as the sum of P uptake in each fraction at each harvest. The amount of P removed from the plots with harvested material was subtracted from the P fertilizer input to estimate the annual P balance. In case of organic fertilization the P input was estimated according to Flisch et al. (2009). In chapter two we showed that the sward P nutrition status was better reflected when plant indicators were applied on the grass fraction rather than on bulk sward samples. Thus, we use P concentration and the P nutrition index (PNI) in the grass fraction as plant P indicators in this study. The PNI was calculated according to Duru and Thélier-Huché (1997):

$$PNI = 100 \times Pm / (0.065 \times Nm + 1.5) \quad [1]$$

with Pm and Nm as the measured P and N concentrations in mg g^{-1} in grasses.

3.3.4. Soil sampling

Soils were sampled 1-2 days after the first regular harvest in May, June and July 2008 in B, W and V, respectively. We took twelve soil cores with an inner diameter of 2.5 cm and 40 cm depth distributed over each plot. Subsequently, soil cores were partitioned into different soil layers and the 12 subsamples were mixed into one composite sample per plot and soil layer. For this study we considered the soil layers 0 to 5 and 20 to 40 cm. The soil was sieved at 2 mm and plant residues were removed. The fresh soil samples were stored at 4°C in the dark for up to two days before extraction with anion exchange resins. Subsequently, soil samples were dried at 40°C for three days and stored at room temperature for later analysis.

3.3.5. Determination of soil P indicators

Ammonium-oxalate extraction

The extraction of P with ammonium oxalate (P_{ox}) was carried out according to Loeppert and Inskeep (1996). Two g of air dry soil were extracted with 80 ml ammonium oxalate solution (ratio 1:40 soil:solution) for 4 hours on an end-over-end shaker in the dark. The ammonium

oxalate solution consisted of 0.1 M oxalic acid and 0.175 M ammonium oxalate. Subsequently, the soil suspension was left standing for 10 minutes and the supernatant was filtered through a 0.2 μm cellulose acetate membrane filter (Sartorius). The filtrate was diluted 1:10 and the P concentration measured by ICP-MS (Agilent, USA). In addition to P, iron (Fe_{ox}) and aluminum (Al_{ox}) concentrations were measured.

Isotopic exchange kinetics

Isotopic exchange kinetics were carried out according to the method described by Frossard and Sinaj (1997). Ten g of dry soil were shaken overhead with 99 ml deionized water in 250 ml bottles (Nalgene) for 16 h to bring the soil solution system at steady equilibrium before the addition of the ^{33}P solution. The bottles were transferred to a magnetic stirrer and continuously agitated for 30 minutes. Afterwards, 1 ml marker solution containing 0.1 to 0.3 MBq carrier free ^{33}P in the form of phosphoric acid (Hartmann Analytik) was added. The soil-water suspension was sampled after 1, 10, 30 and 60 minutes and filtered immediately through a 0.2 μm cellulose acetate membrane filter (Sartorius). One ml of the resulting soil solution was mixed with 5 ml scintillation cocktail (Packard Ultima Gold) and ^{33}P was measured by scintillation counting (Beta counter, Packard A2500, Warrenville, USA). From the isotopic exchange kinetics we derived the slope m of the linear regression between log(r(t)/R) and log(t). Rather than using the measured fraction of total introduced radioactivity remaining in solution after 1 min of isotopic exchange (r_{1min}/R), it was calculated with the regression parameters. The concentration of inorganic P in the soil solution (Cp) was measured by malachite green colorimetry (Ohno and Zibilske, 1991). The quantity of P that is isotopically exchangeable within 1 min (E_{1min}) was calculated (equation 2) as the inverse of the specific activity ($^{33}P/^{31}P$) in the soil solution under the assumption that the specific activity in the soil solution is identical to the specific activity of isotopically exchanged P in the whole system,

$$E_{1\min} = 10 * C_P / (r_{1\min} / R) \qquad [2]$$

with E_{1min} and 10*Cp are given in mg P kg^{-1} soil. To account for analytical variation between dates when IEK were conducted, a control soil (unfertilized and dried soil from W) was included. The isotopic exchange parameters of the control soil were (mean ± standard deviation): Cp= 0.80 ± 0.11 mg kg^{-1}, r_{1min}/R = 0.35 ±0.03, m = 0.42 ± 0.03 and E_{1min} 2.30 ±

0.29 mg kg^{-1}. Because of the low variation between measurement dates we considered the obtained data eligible for comparison.

Resin extraction

Resin-extractable P (P_{res}) was determined after Kouno et al. (1995) by extraction with anion exchange resin membranes that were saturated with HCO_3^-. For extraction 3 g field moist soil were shaken with 30 ml of double-distilled water and two resin strips of 3 x 2 cm (BDH Laboratory Supplies #55164 2S) for 16 h at 150 rpm on a horizontal shaker. After rinsing the membranes with double-distilled water, P was eluted by shaking for 2 h in 0.1 M NaCl/HCl solution. The P concentrations in the eluates were measured colorimetrically as described above. For each soil sample the extraction was done in duplicates and averaged.

3.3.6. Identification of soil P classes

We wanted to classify the sward P nutrition status into the three levels deficient, sufficient and surplus P nutrition (Figure 3.1), respectively. In theory, there is an optimal point of P nutrition ("middle") below which the plant is P deficient and above which P is supplied in surplus (Marschner, 1995). For practical reasons often a range enclosing the optimum value is used to define the sufficient P range. In this study we named the threshold dividing sufficient from deficient as "low" and that dividing sufficient from surplus P nutrition status as "high". The sufficient range is separated into two parts by "middle". The range between low and middle (latent deficiency = no visible symptoms) may reflect a small non-significant risk of reduction of yield and P concentrations. Between "middle" and "high" no penalties on yield and reasonably high P concentrations are to be expected. Above the high threshold, yields are not significantly higher but P concentrations might still increase slightly to the maximum of plant P uptake. These increases in plant P concentration are often connected with large increases in soil P and movement to lower soil horizons. Such relationships are in accordance with the exponential growth response functions developed by Mitscherlich (1928) or the linear plateau model (Waugh, 1973). Both concepts identify a strong response of a plant parameter (e.g. yield or P indicator) to inputs or availability of a nutrient (here P) in the soil. After the maximum, no yield or plant P response is observed, as reflected by the horizontal part of the function. Such a function (with a plateau or maximum) is necessary to identify a surplus P nutrition status. Sufficient or optimum supply is often determined by the economic optimum which is generally

below the maximum. To differentiate deficiency from optimum, critical values are defined above which no deficiency symptoms are observed and yield variance is not significant (e.g. 90% of relative yield).

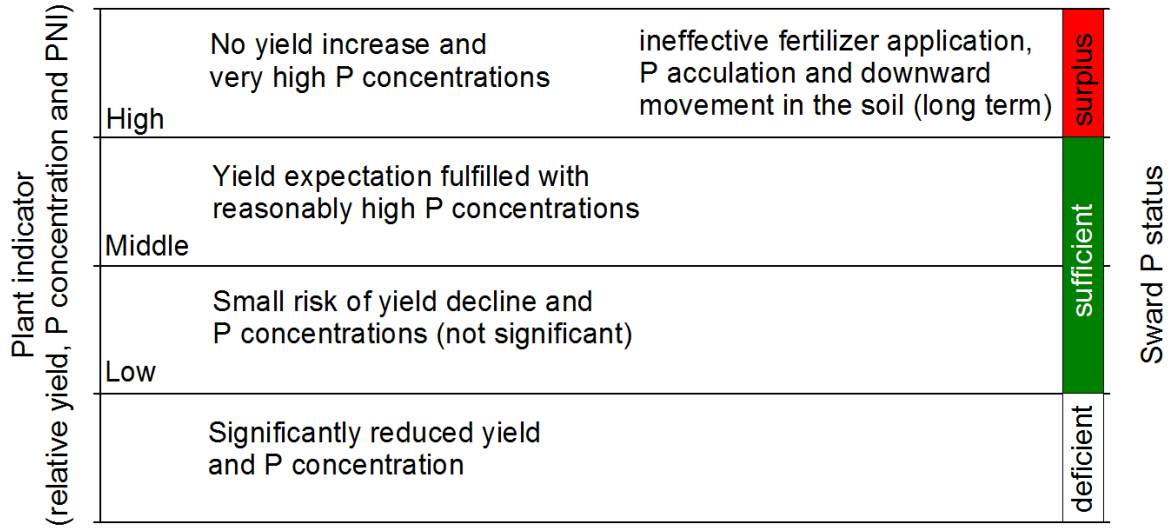

Figure 3.1: Interpretation of plant indicators and their interplay (modified from Kerschberger et al. (1997)). The space between the thresholds "middle" and "high" can be seen as sufficient, whereas between "middle" and "low" the situation is not yet critical but small losses in yield or plant P content cannot be excluded. Below "low" and above "high" significant imbalances between plant P demand and soil P availability exist, thus fertilization in surplus to plant demand or ceased/reduced fertilization, respectively, is necessary.

The plant P nutrition status was determined by yield, relative yield and P uptake, grass P concentration and PNI. In a previous study (Chapter 2), PNI was found to be better correlated to relative yield than P concentration alone. Nevertheless, grass P concentrations can be used when no additional N data is available. Yield, relative yield and P uptake reflect the average output of the total sward. For the relationship between plant and soil indicators we established exponential functions. To derive the thresholds for the soil P indicators we applied plant thresholds derived in the previous study (Chapter 2) to the fitted curve and for the related ± 95% confidence intervals. For relative yield we used the three levels 90%, 100% and the maximum of the response curve. For plant P concentrations and the PNI we used the thresholds of 2.1, 3.0 and 3.5 mg kg^{-1} and 80, 100 and 120 which were shown to be related to 90, 100% and maximum of relative yield (chapter 2). To synthesize the thresholds for each soil P

indicator we selected the highest of the three plant parameters for defining the threshold values of “low”, “middle” and “high”, respectively (Table 3.3).

3.3.7. Statistical analysis

Statistical analyses were performed with R version 2.8.1 (R-Development-Core-Team, 2008). Because experiments of the three locations had different designs, fertilizer treatments were compared between sites only with a F-test. If the F-test was significant at $p<0.01$, a test for least significant difference (LSD) with an alpha of 0.05 was conducted using the library agricolae v. 1.09 (Mendiburu de, 2009). The Holm adjustment for p-values was always applied to control for false positives in multiple comparisons. Prior to analysis, we transformed percentage data of relative yields to their arc sin. Correlation coefficients (ρ) and their level of significance were calculated by the Pearson product-moment correlation. Significance levels are denoted as ns. = not significant, * = $p<0.05$, ** = $p<0.01$ and *** $p<0.001$. Trend lines of the relationships of soil to plant indicators were fitted to graphs in SigmaPlot for Windows version 11.0 (Systat, 2008) using linear and non-linear regression.

3.4. Results:

3.4.1. Investigation of P nutrition status (treatment effects)

Plant P indicators

The selected plant yield and P parameters significantly reflected the long term P fertilization (Table 3.1). Yields increased with the cutting intensity (location). For each location, relative yields greater than 90% were reached when P and K fertilization was close to or higher than P and K uptake, respectively. The treatment differences were also reflected by grass P concentrations and PNI which were significantly lower in treatments omitting P fertilization than in treatments fertilized according to or higher than P uptake. For P uptake the differences between the three locations were even more pronounced than for grass P concentration and PNI.

Table 3.1: Selected plant parameters and annual P balance as affected by location and fertilizer treatment. Treatment differences are shown as least significant differences (LSD) with Holm adjusted p values (alpha = 0.05) for the three locations separately and for all locations together.

Location	Treatment	Total yield	Relative yield[a]	Grass P concentration	Total P uptake	PNI in grasses	Annual P balance[b]
		t ha^{-1}	%	mg g^{-1}	kg ha^{-1}		kg yr^{-1}
Les Verrières	N1P0K0	3.7	74.0	1.7	6.4	62.2	-6.0
(2 cuts)	N1P0.5K0.5	4.7	93.8	2.6	12.4	94.6	-3.4
	N1P1K1	5.2	104.4	3.3	17.3	115.9	1.2
	N1P2K2	5.4	99.1	3.5	18.8	126.3	8.0
LSD		1.3	18.5	0.6	4.6	23.8	4.8
Watt	N0P0K0	3.8	58.6	1.7	6.4	63.7	-8.6
(3 cuts)	N1P0K1	5.1	78.4	1.7	8.9	62.2	-9.5
	N1P1K0	5.3	81.0	3.1	16.3	110.4	-1.0
	N1P1K1	6.9	106.7	3.2	22.4	114.3	-2.4
	N0P1K1	6.6	102.3	3.4	22.3	119.0	-3.2
	N1P1K2org	6.4	99.1	2.4	15.4	86.3	2.1
LSD		1.3	19.7	0.5	6.2	22.9	6.2
Baldegg	N1P1K2org	11.2	97.3	4.1	46.1	132.1	-4.6
(5 cuts)	N1P1K2org/min	14.7	109.2	3.9	57.9	119.1	-14.3
	N2P2K1org I	13.8	102.4	4.5	62.4	135.4	84.8
	N2P2K1org II	13.8	101.9	4.3	59.4	129.0	83.0
LSD		1.9	14.8	0.4	12.1	10.9	12.1
LSD total		1.8	20.6	0.6	9.7	21.6	9.7

[a] calculated as the percentage of the yield indication for the respective location from Flisch et al. (2009), see also table 2.3

[b] calculated as input output balance on the field scale, with organic fertilizer input estimated according to Flisch et al. (2009)

Soil analysis

Similar to the plant analysis the long term P fertilization treatments were significantly differentiated by the three soil P indicators (Table 3.2). The total range was 141 to 908, 1.5 to 73 and 0.5 to 69 mg P kg^{-1} dry soil in the 0 to 5 cm layer for P_{ox}, E_{1min} and P_{res}, respectively. For P_{ox}, values were highest in B and lowest in W, whereas for E_{1min} V and W were in a similar range but lower than B. Although P_{res} had similar values for unfertilized treatments in V and W, the norm fertilized treatment (N1P1K1) had twice as much P_{res} in V as in W. Notably, for N1P1K1org in W we extracted as low P_{res} as for the two unfertilized treatments N0P0K0 and N1P0K1. For each of the three soil P indicators, the highest values were extracted in the treatments N2P2K1org I and II in B. The overfertilized treatment N1P2K2 in V was higher than the two treatments fertilized below recommendation (N1P0K0 and N1P0.5K0.5) but similar to N1P1K1, except for P_{res}.

In 20-40 cm soil depth, treatment differences were generally much smaller than in 0-5 cm and mostly not significant in V and W. For P_{ox} in B, N2P2K1org I and II were still significantly higher than N1P1K2org. No significant treatment differences were observed for E_{1min} in any of the sites. For P_{res} the surplus fertilized treatments were still significantly higher than the other treatments.

Table 3.2: Mean P extracted with ammonium oxalate (P_{ox}), P exchanged in one minute (E_{1min}) and resin extractable P (P_{res}) as affected by location and fertilizer treatment in the soil layers 0-5 cm and 20-40 cm. Treatment differences are shown as least significant differences according to a LSD test with Holm adjusted p values (alpha = 0.05) for the three locations separately and for all locations together.

Location	**treatment**	**0-5 cm**			**20-40 cm**		
		P_{ox}	**E_{1min}**	**P_{res}**	**P_{ox}**	**E_{1min}**	**P_{res}**
			mg kg^{-1}			mg kg^{-1}	
Les Verrières	N1P0K0	242	4.3	1.3	113	4.3	0.4
(2 cuts)	N1P0.5K0.5	271	4.5	7.0	107	5.0	0.6
	N1P1K1	304	4.9	15.3	115	3.5	0.5
	N1P2K2	384	6.5	33.4	127	3.3	1.2
LSD		111	1.7	7.5	24	4.1	0.6
Watt	N0P0K0	168	2.7	1.1	175	0.8	1.0
(3 cuts)	N1P0K1	195	2.1	1.0	174	1.3	0.8
	N1P1K0	223	9.7	9.6	188	1.0	1.5
	N1P1K1	227	4.9	7.4	197	1.4	1.0
	N0P1K1	219	5.3	7.0	192	1.2	1.3
	N1P1K2org	192	7.6	1.1	226	1.3	0.9
LSD		89	3.6	4.9	64	0.7	0.8
Baldegg	N1P1K2org	403	17.1	11.3	171	1.6	2.2
(5 cuts)	N1P1K2org/min	471	12.5	11.8	242	1.9	3.4
	N2P2K1org I	743	47.5	58.6	336	6.1	19.4
	N2P2K1org II	840	57.0	54.5	429	7.0	15.8
LSD		91	25.2	9.3	125	13.6	10.4
LSD total		112	17.2	8.6	74	3.2	6.8

3.4.2. Relationship of soil to plant indicators

Yield and P uptake

We observed positive site-specific relationships between the soil P indicators and plant yield and P uptake (Figure 3.2). The coefficient of correlation (ρ) was always higher for P uptake than for yield. Among the soil P indicators, ρ for P_{ox} was the highest and for P_{res} the lowest. Because of the different yield levels at each location we fitted linear trend lines individually for each location. These relationships were always significant in V, with the strongest relation observed for P_{res} and the weakest for E_{1min}. In W, significant relationships were found between yield and P_{res} and between P uptake and E_{1min} and P_{res} (Figure 3.2). For B no significant increase of yield and P uptake with increasing soil P was observed. However, a global linear fit (using the locations together) resulted in r^2 of 0.64, 0.50 and 0.46 for yield and 0.72, 0.54 and 0.46 for P uptake with P_{ox}, E_{1min} and P_{res}, respectively. In contrast to the individual relationships, P_{res} was weaker than P_{ox} and E_{1min} for the global relationships to yield and P uptake. In P_{res}, there was a clearer differentiation between the P fertilization treatments.

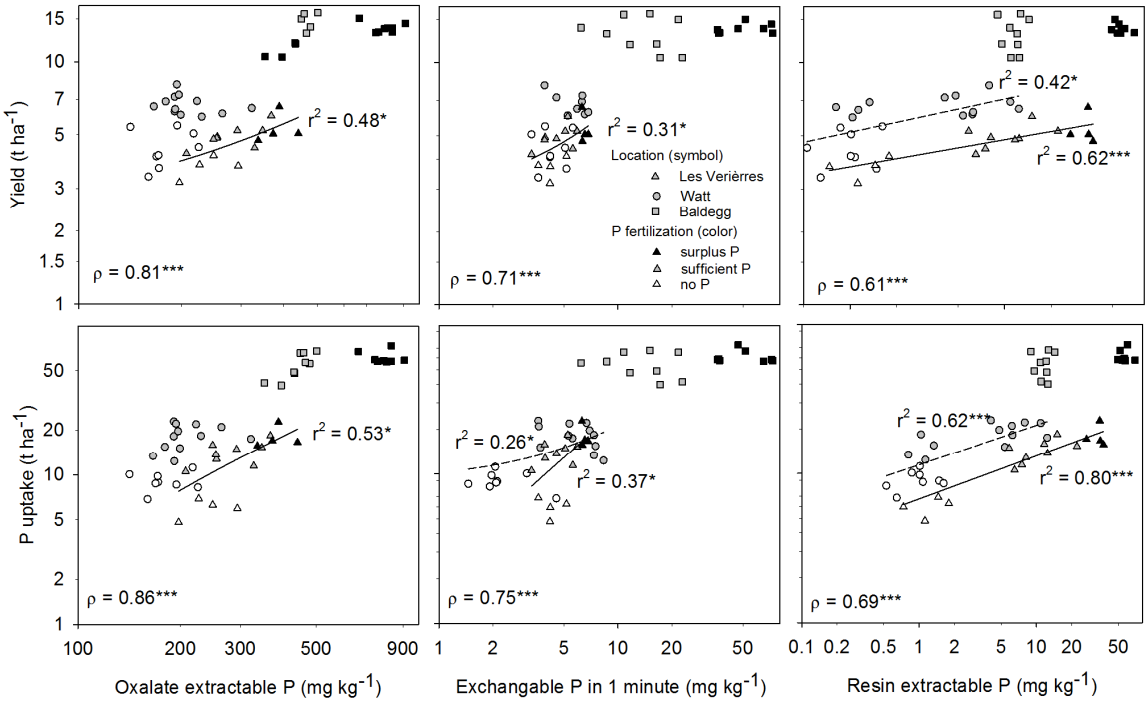

Figure 3.2: Relationships of yield (aboveground dry matter) and P uptake with the three soil P indicators oxalate extractable P, P exchangeable in 1 min and resin-extractable P. Different symbols and colours indicate the three different harvest intensities and the three classes of P fertilization, respectively. For the relationships the Pearson correlation coefficient (ρ) is shown. Linear trend lines were fitted (y=y0+ax) for each location (management) separately. Significance levels are: not significant, * = p<0.05, ** = p<0.01 and *** = p<0.001, trend lines without indicated r^2 are not significant.

Relative yield, P concentration and PNI in grasses

Relative yield, P concentrations and PNI were positively related to P_{ox}, E_{1min} and P_{res} (Figure 3.3). In contrast to the relationships with yield and P uptake, these relationships were stronger when the three locations V, W and B were used in combination. The relationships between plant indicators and soil P extractions were well described as a rise to maximum function. Grass P concentration and PNI were better correlated to the soil indicators than the relative yield. The amount of P_{res} was better correlated to the plant indicators than E_{1min} and P_{ox}.

Below P_{ox} of 300 and E_{1min} of 10 mg P kg^{-1}, plant P concentrations and PNI varied much more than above these values (Figure 3.3). Below these values we observed a variation by 2.3 mg g^{-1} and 85, for P concentrations and PNI, respectively. Above the variation was reduced to 1.8 for

P concentration and 40 for PNI. In contrast, the variation in plant P concentration and PNI was not higher than 1.4 mg P kg^{-1} and 50 over the whole range of P_{res} observed in the soil.

3.4.3. Identification of thresholds

The three plant indicators resulted in slightly different thresholds for the three P nutrition status levels (Table 3.3). At the low threshold, relative yield was related to higher values for each of the soil P indicators than grass P concentration and PNI. At the middle threshold the three plant indicators were related to similar ranges of soil P. At the high threshold grass P concentration and PNI resulted in higher values of soil P indicators than relative yield. The maximum of the fitted curve reflected values of relative yield, grass P concentration and PNI that were marginally higher than the high plant threshold. In contrast, the corresponding soil P indicators continued to increase substantially beyond the high soil P threshold (Table 3.3). Given the relative changes of P nutrition status among the plant indicators a combination for the identification of soil P status seems practical. Using always the highest plant indicator for each threshold level ensures that the other two reach their required level as well.

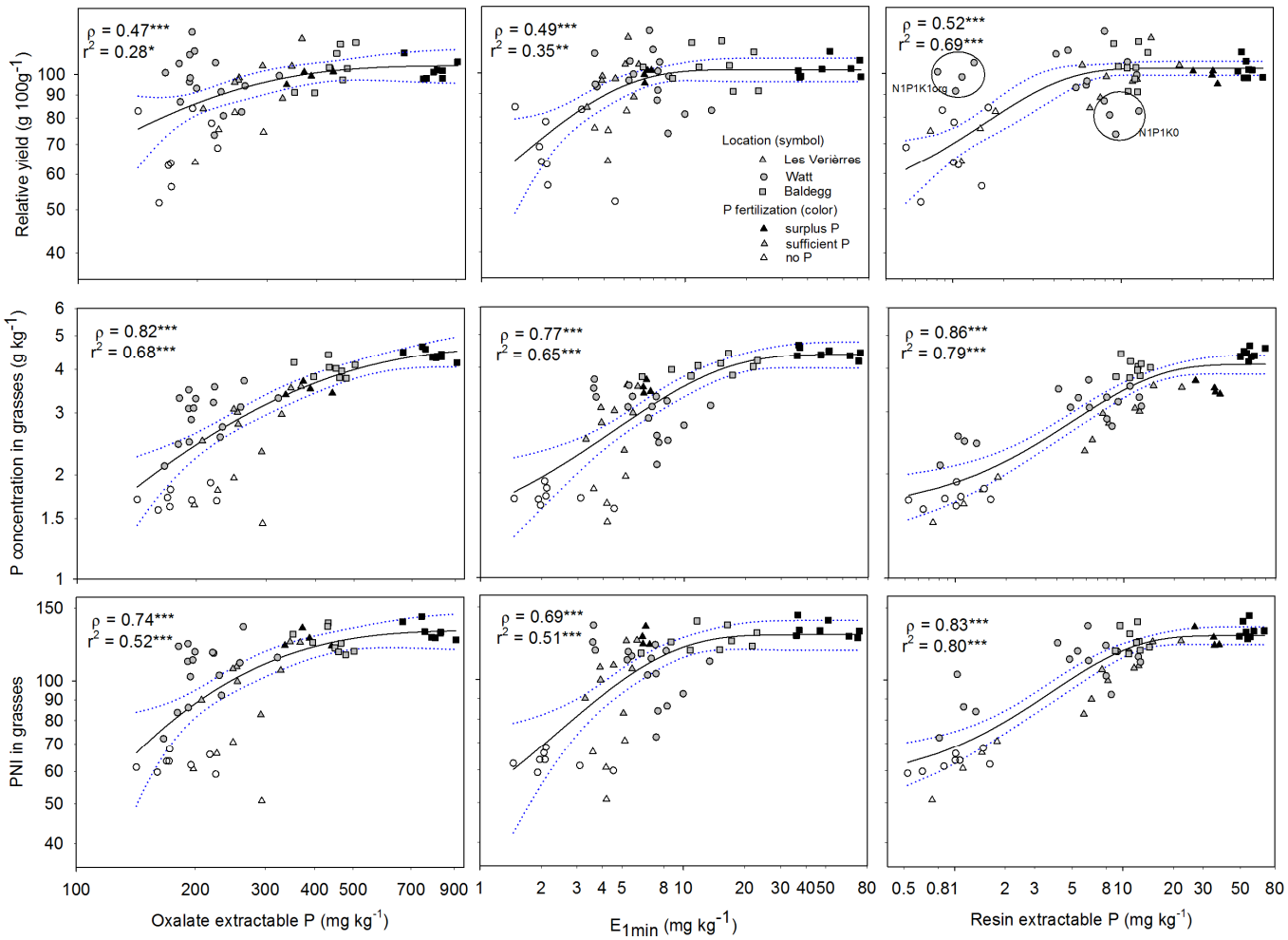

Figure 3.3: Relationship of relative yield, P concentration in grasses and PNI in grasses with the three soil P indicators oxalate extractable P, P exchangeable within 1 min, and resin-extractable P. Different symbols and colours indicate the locations and the three classes of P fertilization, respectively. For the relationships the Pearson correlation coefficient (ρ) is shown. Trend lines were fitted as exponential rise to maximum ($y=y0+a\times(1-e^{(-b\times x)})$). Significance levels are: * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

Table 3.3: Mean and ± 95% confidence ranges of P_{ox}, E_{1min} and P_{res} as related to four levels of relative yield, P concentration and PNI. The relationship is reflected by the function $y = y0+a*(1-e^{(-b*x)})$, with y = plant indicator and x = soil P extract. For the maximum of the function no 95% confidence range is shown. The value at which the maximum is reached is shown in brackets. For the synthesis of the threshold derived by the three plant parameters the highest observed value was selected.

Plant indicator		**P_{ox}**		**E_{1min}** mg kg^{-1}		**P_{res}**	
	Total range	141-908		1.46-73.2		0.53-69.3	
	CV	58		131		120	
	Level	**mean**	**± 95%**	**mean**	**± 95%**	**mean**	**± 95%**
Relative yield							
Low	90%	230	+190 -290	4	+3.2 -5.4	3	+2.0 -4.5
Middle	95%	280	+225 -375	5.7	+4.0 -7.5	4	+2.5 -6.0
High	100%	380	+275 –aor*	8.75	+5.5 -aor	6	+3.2 –aor
	maximum	540 (103%)		18 (102%)		9 (102%)	
P concentration							
Low	2.1	165	+aor -190	2.5	+aor -3.3	1.8	+1.0 -2.4
Middle	3	270	+250 -310	6.5	+5.3 -7.5	6	+5.0 -7.3
High	3.5	360	+320 -420	9.8	+8.0 -12.0	10.2	+8.6 -12.6
	maximum	aor		30 (4.3)		24 (4.1)	
PNI							
Low	80	175	+aor -195	3.1	+1.8 -3.5	2.4	+1.6 -2.6
Middle	100	250	+220 -290	5.1	+4.2 -6.3	5	+3.9 -5.9
High	120	380	+310 -510	9.8	+7.6 -aor	10.2	+8.6 -14
	maximum	700 (131)		23 (128)		23 (128)	
Thresholds							
Low		**230**	+190 -290	**4**	+3.2 -5.4	**3**	+2.0 -4.5
Middle		**280**	+250 -375	**6.5**	+5.3 -7.5	**6**	+5.0 -7.3
high		**380**	+320 -510	**9.8**	+8.0 -12.0	**10.2**	+8.6 -14

*aor = out of range

3.5. Discussion

3.5.1. Relationship between soil and plant P indicators reflects long term P fertilization

The treatment effects on the investigated plant and soil P indicators illustrate successfully deficient, sufficient and surplus P conditions. These treatment effects are confirmed by strong coefficients of correlation for plant to soil relationships.

The shapes of the relationships of the three soil P indicators suggested no major differences besides magnitude of soil P. To identify soil P status classes for P_{ox}, E_{1min} and P_{res} we used a curve fitting approach instead of boundary lines (Schnug and Heym, 1996) or Cate-Nelson quadrants (Cate and Nelson, 1971). Using Boundary lines or Cate-Nelson quadrants would generally identify a lower or a higher threshold, respectively (Breuer et al., 2003). The boundary line approach identifies the first value when a defined level is reached (e.g. 100% relative yield) as the threshold. In contrast, Cate-Nelson quadrants identify the threshold as the separation line between a low-yielding and a high-yielding sample population using the highest value of the low-yielding population as the fix point. The curve fitting approach can be interpreted as intermediate and the 95% confidence range can be used to take the variation into account (Table 3.3).

3.5.2. Thresholds derived for soil P indicators

Using the highest among the three plant indicators ensured that a given threshold value was valid for each of the three plant indicators (Table 3.3). Using only one of the plant indicators to derive the threshold would neglect the differences between the plant indicators and lead to under or overestimated threshold values. Thus, a combination of yield and P nutrition status indicators should be used if possible.

The high threshold, representing 100% relative yield, a grass P concentration of 3.5 mg P kg^{-1} and a PNI of 120 proved to be applicable as the threshold indicating surplus P nutrition. In other studies often the maximum yield response was used for that purpose (e.g. Mallarino and Atia, 2005). In our study the plant indicators in treatments above the high threshold were never statistically different from treatments above the maximum of the response curve. For relative yields the difference between “high”

(100%) and maximum (102-103.5%) was related to substantial differences in the assigned thresholds of the soil P indicators (Table 3.3). For grass P concentration and PNI theses differences (high to maximum) induced large changes in the corresponding soil P indicator (Table 3.3). Using the point of maximum response would include a considerable range of ineffective soil P. Thus, the use of the high threshold of plant P indicators to define surplus P nutrition may improve the identification of the surplus P nutrition status in the soil.

Thresholds for oxalate extracted phosphorus

According to the plant indicators we classified P_{ox} below 230 (low) and above 380 mg kg^{-1} (high) soil as deficient and surplus P supplied, respectively. Keller and van der Zee (2004) reported a range of P_{ox} under intensive managed grassland in Switzerland of 268 to 1226 mg P kg^{-1}. They related P_{ox} to P extracted with ammonium-acetate EDTA (P_{AAE}) which is a standard soil P analysis in Switzerland. We used thresholds derived for P_{AAE} in our study (chapter 4) to extrapolate P_{ox} from the data reported in Keller and van der Zee (2004). The middle and high thresholds were reached approximately at 300 and 400 mg P kg^{-1}, respectively. Although they did not propose thresholds their data supports the thresholds we derived in our study.

Koopmans et al. (2004) reported a range of P_{ox} between 470-925 mg kg^{-1} in a P mining study over 978 days using non calcareous sandy soil. In contrast to our findings, they observed P deficiency in *Lolium perenne* when soil P_{ox} fell below 640 and 680 mg P kg^{-1} soil for pots filled with 5 and 10 cm soil, respectively. This value is much higher than what we observed in the field. It exceeds even the 95% range of 510 mg P kg^{-1} which practically excludes P deficiency in our experiment. The restricted root growth in the pot experiment might be an explanation why the relatively high P_{ox} was not sufficient for the plants. Although we observed most root biomass in the top 10 cm in the field, at least 40 cm of the soil were accessed by roots (personal observation).

A higher range of P_{ox} (310-1240) was reported by Bell et al. (2005b) for basaltic and non-basaltic soils under grassland. However, they found a stronger relationship between herbage P nutrition status (identified as DRIS P) and the degree of P saturation (DPS) instead of P_{ox}. That is in contrast to our findings where the DPS was always weaker correlated to plant P nutrition status than P_{ox} (data not shown). That was also

true when a DRIS P index was calculated (data not shown). Nevertheless, Bell et al. (2005) did not report negative DRIS P in their study, indicating that above P_{ox} of 310 no severe P limitation was observed. Since it was in the range we defined as the middle threshold that supports our finding as well.

The phosphorus exchanged in one minute

We found severely decreased yields (below 90%) when E_{1min} was below 4 mg P kg^{-1}, with the corresponding 95% range of 3.2 to 5.4 mg P kg^{-1}. That is in the range of the finding of Gallet et al. (2003a) who observed a reduced crop production below E_{1min} of 5 mg P kg^{-1}. This observation was based only on one trial where they found a yield response. Thus, they proposed it as a preliminary threshold. However, it is in the same range as the values reported by Morel et al. (1992) for wheat.

For grassland Gallet et al. (2003) reported increasing plant herbage P concentrations until E_{1min} of 18 mg kg^{-1}, but without a yield response. Such a value can be considered as the maximum of the response curve. It is much higher than the high threshold of 10 mg kg^{-1} which we propose. The maximum points observed in our study were equal or higher than 18 (Table 3.3). However, the response curve of E_{1min} with grass P concentrations (Figure 3.3) had a higher maximum than relative yield and PNI (Table 3.3). Using only grass P concentration to identify surplus P nutrition status resulted in a higher threshold. Further, we found no treatment differences between relative yield and PNI above E_{1min} of 6.5 which is in agreement with the E_{1min} of 6.0 mg P kg^{-1} suggested for wheat by Morel et al. (1992). Thus, we assume that the range above the high threshold of 10 mg P kg^{-1} largely reflects surplus P nutrition. This is in line with findings by Tran et al. (1988). In a pot experiment they found E_{1min} above 10 mg P kg^{-1} only to reflect relative yields close to maximum for *Lolium multiflorum*, but plant P uptake still increased in two of three investigated soils above E_{1min} of 10 mg P kg^{-1}.

Resin extractable P

He et al. (1997) suggested that a combined interpretation of the resin-extractable and microbial P pool might give a better indication of the potential plant P availability in the field. But we know of no study which determined P nutrition status classes for permanent grasslands based on P_{res} extracted from field moist soils.

However, some studies have used the resin-extraction on dry soil to determine P availability (Qian et al., 1992). For example, Chen et al. (2000) found an average P_{res} of 5.2 mg P kg^{-1} under permanent grassland, which refers to a sufficient P availability in our classification (Table 3.3). Mallarino and Atia (2005) and Cantarella et al. (1998) reported similar ranges of P_{res} as in our study for corn and perennial crops (grassland), respectively. Mallarino and Atia (2005) found a maximum yield response of corn at P_{res} of 13 and 19 mg P kg^{-1} using the Cate-Nelson or linear plateau procedure, respectively. For our function, the maximum relative yield was reached at a P_{res} of 13 mg P kg^{-1} as well. In Cantarella et al. (1998), a medium P nutrition status class is defined as between 10 and 21 mg P kg^{-1} (recalculated for the average soil density of 1.2 g cm^{-3} observed in our study). This range appears to be higher than what we found. However, our results were obtained from field moist samples and thus cannot be compared directly to P_{res} extracted from dried soil. The extraction of dried soil (soil moisture < 1%) of the treatment N0P0K0 in W revealed a tenfold increase of P_{res} (moist: 1.1± 0.3, dry 10.1±0.5 mg P kg^{-1}). This release of P probably originated largely from the microbial biomass (Bünemann, personal communication and chapter 5). Such an increase of P_{res} by soil drying may explain the higher thresholds or classes found by Cantarella et al. (1998).

3.5.3. Soil P indicators have different effectiveness to reflect P nutrition status

Although the shapes of the response curves were similar, the three soil P indicators showed different accuracy in identifying plant P nutrition status. Below the thresholds for P_{ox}, E_{1min} and P_{res} of 290, 5 and 4 mg P kg^{-1}, respectively, we found P fertilized and unfertilized treatments. However, these treatments were clearly differentiated by yield and plant P parameters (Figure 3.2 and 3.3). Thus, values below 290, 5 and 4 mg P kg^{-1} for P_{ox}, E_{1min} and P_{res}, respectively, cannot be reliably interpreted as clearly P deficient and vice versa. This was most pronounced for P_{ox}. There we observed P fertilized treatments with high yield and plant P indicators over the same range of P_{ox} values as unfertilized treatments with reduced yield and P nutrition status. For E_{1min} we found such an uncertainty only in the 95% range of the low threshold. Below that range we found only N1P0K1 where P fertilization was omitted since 16 years. Thus, it was the

only treatment in our study which was severely P limited and not co-limited by K or N. Such a co-limitation might have decreased the soil P over time more than a solitary P limitation because of higher sward P uptake. In contrast to P_{ox} and E_{1min}, we found P_{res} below 2 mg P kg^{-1} only in N1P1K2org. For N1P1K2org a risk for reduced yield and a lower P nutrition status was indicated by the plant indicators. This suggests a better reflection of the sward P nutrition status by P_{res}, which was measured on moist soils, because the plant P status was reflected with less uncertainty. Nevertheless, a practical interpretation is that P deficiency was excluded for all P indicators when the value at the lower 95% confidence level was used.

Additionally we found the P status of the fertilizer treatments differently reflected by the soil P indicators. For example, N1P0.5K0.5 and N1P1K1 had nearly similar E_{1min} (Table 3.2) but were significantly differentiated by grass P concentration and PNI (Table 3.1). N1P0.5K0.5 tended to have reduced yield whereas N1P1K1 in 2008 had yields above indication. This observation illustrated on the one hand that different pools of P were extracted by P_{ox}, E_{1min} and P_{res}. On the other hand, plant P indicators were superior when P_{ox} and E_{1min} were below 290 and 5 mg P kg^{-1}, respectively. N1P1K1org was the only P fertilized treatment with P_{res} below 4 mg P kg^{-1}. The corresponding yield, P concentration and PNI were higher than in the P unfertilized treatments but did not reach the level of the mineral P fertilized treatments in W. In another study (Chapter 5) we found strong variation of P_{res} and P_{mic} in W. In N1P1K2org, P_{mic} was always significantly higher than in N1P1K1 and N1P0K1 in W. In addition, the observed decrease of P_{res} when P_{mic} was increased after harvests was most pronounced in N1P1K2org. In this treatment we observed an average P_{res} over the whole season of 4.6 mg P kg^{-1} in 2009. Since the soil samples were taken after harvests in the present study this observation can explain the low P_{res} found despite of higher relative yield and P status. That underlines the importance of the sampling time when P_{res} is analyzed on field moist samples.

3.6. Conclusion

Due to the wide range of P input treatments over a long period and different management intensities we observed plant P responses from severely deficient to clearly surplus P supplied. To identify thresholds for soil P indicators in terms of plant

P availability we suggest using a set of plant indicators. These indicators should reflect yield and P status response by the best available indicator (e.g. PNI in the grass fraction for grasslands). To identify P surplus availability we suggest using the high thresholds for the plant indicators, instead of the maximum of the response curve. We found thresholds dividing deficient, sufficient and surplus P availability classes of 290 and 510 mg P kg^{-1} for P_{ox}, 5.4 and 12.0 mg P kg^{-1} for E_{1min} and 4.5 and 14 mg P kg^{-1} for P_{res}. Among the soil P indicators P_{res} had the strongest relationship to the plant indicators and provided a better reflection of the P fertilizer treatments in our study. At low range the P status was better indicated by the plant P indicators grass P concentration and PNI, and by the soil P indicator P_{res} than by P_{ox} and E_{1min}. However, at higher soil P levels the three soil indicators allowed a better differentiation of the fertilizer treatments than the plant P indicators.

Chapter 4: Thresholds for the Swiss routine soil phosphorus tests in agricultural grassland

4.1. Introduction

The Swiss reference methods for routine tests to estimate phosphorus (P) availability in the soil are the chemical extractions with CO_2-saturated water (Dirks and Scheffer, 1930) and ammonium-acetate EDTA (FAL et al., 1996, modified from Cottenie et al., 1982). Hereafter, P extracted with these methods will be named as P_{CO2} and P_{AAE}, respectively. The extraction with CO_2-saturated water works at low ionic strength close to soil pH but is sensitive to changes of experimental conditions during extraction (e.g. temperature). Ammonium-acetate EDTA is an acid in combination with a complexing agent. It is less affected by experimental conditions during extraction. However, the determination of available P with P_{AAE} was reported to be stronger affected by soil properties such as pH and carbonate content than P_{CO2} (Stünzi, 2011). Demaria et al. (2005) found P_{CO2} to underestimate rapidly exchangeable P, and P_{AAE} to additionally extract significant amounts of P which may not be available to plants. Extraction of unavailable P with P_{AAE} increased with increasing pH. This underlines that P_{AAE} is a mixture of P forms from the soil which are not well defined for their P availability to plants. For fertilizer recommendation, P_{CO2} and P_{AAE} are interpreted by taking into account the clay and organic matter content of the soil. For specific soil types parent material is considered (Flisch et al., 2009).

The accumulation of P in agricultural soils and related P losses to the environment are of growing concern. Both P accumulation in soils and losses to the environment reflect an inefficient P management. An underestimation of P availability by soil P testing was given as one reason for P accumulation observed in western European countries (Schröder et al., 2010). Thus, a re-evaluation of soil interpretation schemes considering yield and potential environmental impacts was recently suggested by Römer (2009) and Schröder et al. (2010). Neyroud and Lischer (2003) suspected an underestimation of soil P availability by the classification of P_{CO2} and P_{AAE} with the Swiss interpretation scheme. A similar conclusion was drawn by Frossard et al. (2004) who synthesized data from many long term fertilizer trials in Switzerland. Gallet et al. (2001) reported

thresholds below which P availability might limit the yield of P demanding crops (e.g., potato) as 0.5 and 35 mg P kg^{-1} for P_{CO2} and P_{AAE}, respectively. Since they found no yield limitation in their investigated grasslands they could not propose thresholds for yield limitation in grasslands.

The objective of this study is to evaluate the relationships of yield, grass P concentration and P nutrition index (PNI) to P_{CO2} and P_{AAE}. Following the approach developed in chapter three we establish thresholds to define a deficient, sufficient and surplus soil P status for permanent grasslands under agricultural use in Switzerland. The investigated grasslands differed in cutting intensity and applied fertilizer treatments with no P, sufficient P and surplus P.

4.2. Material and Method

Soils were sampled from the three locations Les Verriéres (V), Watt (W) and Baldegg (B) and processed as described in chapter 3.3. For the soil analyses we used the 0-5 cm soil layer. This contrasts with the recommendation of 0 to 10 by the Flisch et al. (2009). The extraction of P_{CO2} and P_{AAE} was done according to the guidelines given for the reference methods (FAL et al., 1996) except that the P concentration in the extracts was measured using malachite green colorimetry (Ohno and Zibilske, 1991). Subsequently, we classified the soils according to Swiss soil P status classes (Flisch et al., 2009) using the average clay contents for each of the three locations (Table 2.2). The soil P status classes in Flisch et al. (2009) range from A to E, with A being deficient and E surplus for plant P supply. Class C refers to sufficient P availability in the soil and results in a recommended P fertilization similar to sward P uptake. However, the analysis was not done in a certified lab for soil routine analysis, thus data should be interpreted with care. Nevertheless the values measured in V and W were in agreement with results observed from Agroscope Changins-Wädenswil (ACW) and Agroscope Reckenholz-Tänikon (ART) which managed these fertilizer trials, respectively (Huguenin-Elie and Jeangros, personal communication). In the on farm grasslands in B the values were close to the values from a certified lab (Labor für Boden und Umweltanalytik, Thun) which were measured 2 to 4 years before.

Plant indicators used were relative yield, grass P concentration and the grass PNI (measurement and calculation explained in chapter 2). We established exponential

functions with a maximum for the relationships between the plant indicators and P_{CO2} and P_{AAE}. Subsequently, we applied the three plant thresholds low, middle and high to establish thresholds for the three soil P status classes deficient, sufficient and surplus. The plant indicators and the procedure were described in detail in chapter three (see figure 3.1).

We define the P nutrition status as P deficient when plant growth is significantly lower than the expected yield for a given cutting regime. To define a yield expectation we used the yield indication given in Flisch et al. (2009) for a range of cutting regimes and altitudes. At a sufficient P nutrition status plant yield fulfils the indicated yield and an surplus P fertilization would increase soil P availability but not the plant yield. The surplus P nutrition status defines a situation where P fertilizer input has no yield response.

4.3. Results

4.3.1. Treatment differences

For the plant parameters total sward yield, relative yield, grass P concentration, sward P uptake and the PNI the differences between locations and treatments were shown in chapters two and three. Similar to the plant P indicators (chapter 2) and the other investigated soil indicators (chapter 3), P_{CO2} and P_{AAE} reflected the long term P fertilization. P_{CO2} and P_{AAE} ranged from 0.17 to 22.1 and 3.6 to 322 mg P kg^{-1}, respectively, in the 0 to 5 cm soil layer. The results for the soil layers 5 to 10, 10 to 20 and 20 to 40 can be found in Table A.9 (Annex).

For P_{CO2} the values were highest in B while the values in V and W were in a similar range. Significant differences between treatments were mainly found in V and B (Table 4.1). In V the treatments fertilized according to or higher than average sward P uptake had significantly higher P_{CO2} than at lower or no P fertilization. In B, the overfertilized treatments had higher P_{CO2} than the two treatments fertilized according to recommendation. In W, only in N1P1K0 P_{CO2} was significantly higher than the P unfertilized treatments (N0P0K0 and N1P0K1), although P fertilized treatments tended to be higher than P unfertilized treatments (Table 4.1). The P_{AAE} values were also highest in B, but lowest in W (Table 4.1). Additionally the treatment differences in W

were more distinct than with P_{CO2}. In B the overfertilized treatments were significantly higher than the other two.

4.3.2. Soil P classes

The soil P status classes largely reflected the treatment differences found for P_{CO2} and P_{AAE}. But both methods resulted in different P nutrition status classes except for the treatment fertilized in surplus to average sward P uptake (Table 4.1). In V, P_{AAE} resulted in a soil P status classes from A to C, with N1P1K1 and N1P2K2 classified as C. The range of P_{CO2} in V was from class B to C, with only N1P2K2 classified as C. In W the two methods showed contrasting results: whereas P_{CO2} put the P unfertilized treatments in class B and the P fertilized treatments in class C, all soils in W were classified as A for P_{AAE}. In B the two treatments N1P1K1org and N1P1K2org/min were always ranked two classes higher by P_{CO2} than by P_{AAE}. The overfertilized treatments in B were found in the highest class E for both soil P extractions (Table 4.1).

Table 4.1: Mean P_{CO2} and P_{AAE} as affected by location and fertilizer treatment in the soil layer 0-5 cm. Treatment differences are shown as least significant differences according to a LSD test with Holm adjusted p values (alpha = 0.05) for the three locations separately and for all locations together. Correction factor for fertilizer recommendation and the P nutrition status class were determined according to Flisch et al. (2009). The P nutrition status classes range from A to E, with A being poor and E enriched in P. Class C refers to sufficient P availability in the soil and recommendation of P fertilization equivalent to sward P uptake.

Location	**Treatment**	**P_{CO2}**			**P_{AAE}**		
		mg kg^{-1}	correction factor	P nutrition status class	mg kg^{-1}	correction factor	P nutrition status class
Les Verrières	N1P0K0	0.20	1.3	B	6.2	1.5	A
	N1P0.5K0.5	0.25	1.3	B	---[a]	---	---
	N1P1K1	0.39	1.2	B	28.6	1.0	C
	N1P2K2	0.55	1.1	C	43.2	1.0	C
	LSD	0.17			23.4		
Watt	N0P0K0	0.37	1.3	B	4.4	1.5	A
	N1P0K1	0.34	1.3	B	4.9	1.5	A
	N1P1K0	1.26	1	C	19.4	1.5	A
	N1P1K1	0.84	1.1	C	12.0	1.5	A
	N0P1K1	0.86	1.1	C	11.8	1.5	A
	N1P1K2org	0.96	1	C	7.1	1.5	A
	LSD	0.71			6.9		
Baldegg	N1P1K2org	4.60	0	E	55.3	1.0	C
	N1P1K2org/min	1.77	0.8	D	31.3	1.2	B
	N2P2K1org a	16.70	0	E	238.6	0.0	E
	N2P2K1org b	10.70	0	E	239.3	0.0	E
	LSD	10.03			88.4		
	LSD total	6.77			61.5		

[a] not detected

4.3.3. Relationship of P_{CO2} and P_{AAE} to the plant indicators

We observed positive relationships to all plant indicators shown in chapter three (Table 3.1), except for yield and P uptake in B. Since yield and P uptake were not used to derive thresholds for the soil P indicators in chapter three they are not shown here. The relationships of P_{CO2} and P_{AAE} with relative yield, P concentration and PNI were well described by exponential functions with a maximum (fitted curve). For P_{AAE}, the relationships and the coefficient of determination for the fitted curve were stronger when the locations were combined (Figure 4.1) than for individual locations. In contrast, for P_{CO2} the relationships were stronger when V was investigated individually

and W and B were combined (Figure 4.2). The two groups V and W+B refer to soil clay contents of about 30% and 16 to 26%, respectively (Table 2.2).

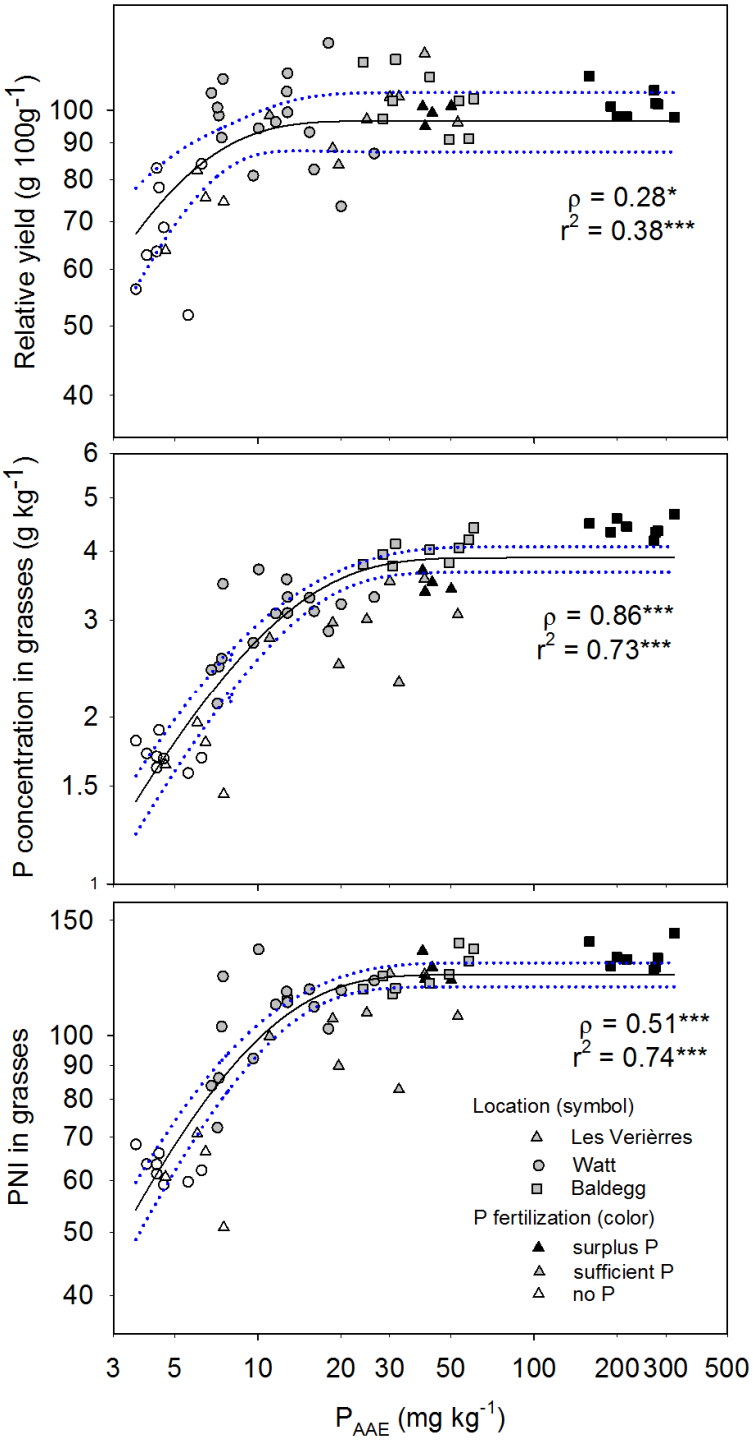

Figure 4.1: Relationship of relative yield, P concentration and PNI in grasses with P_{AAE}. Different symbols and colours indicate the three locations and the three classes of P fertilization, respectively. For the relationships the Pearson correlation coefficient (ρ) is shown. Trend lines were fitted as exponential rise to maximum ($y=y0+a\times(1-e^{(-b\times x)})$). Significance levels are: * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

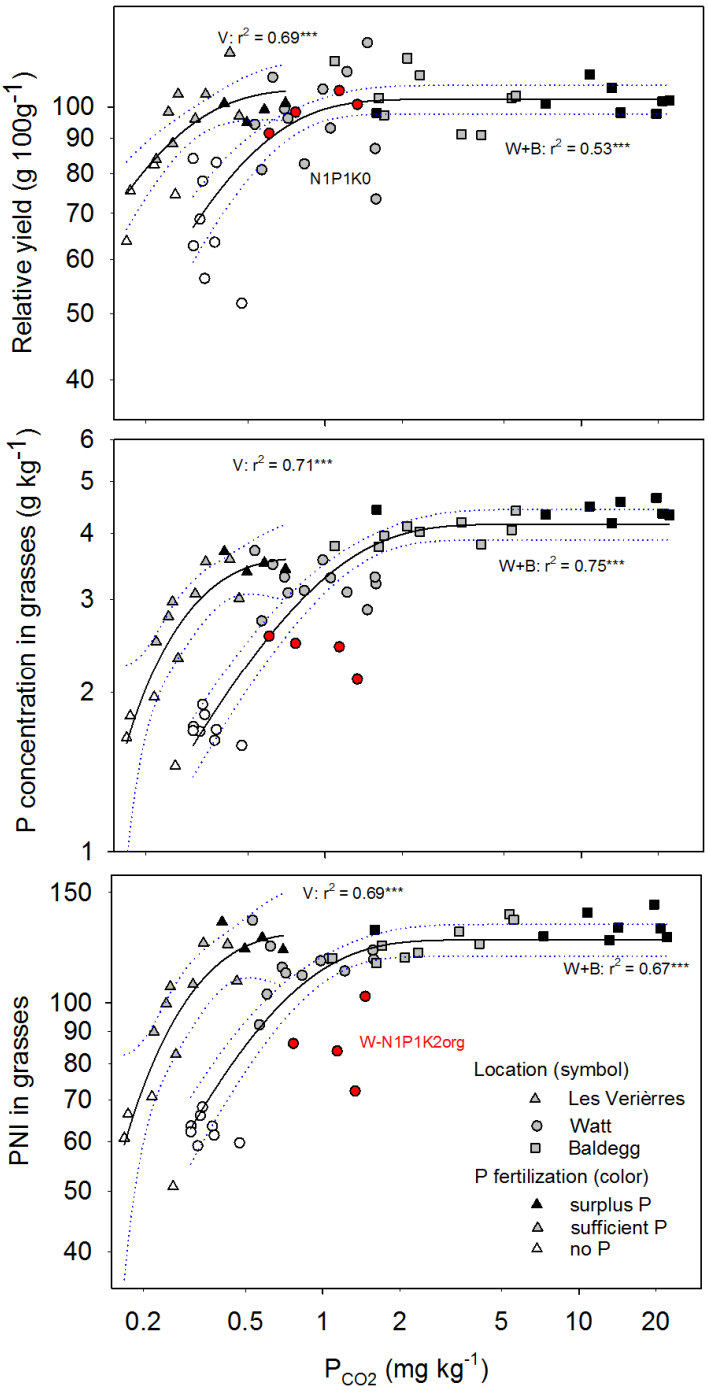

Figure 4.2: Relationship of relative yield, P concentration and PNI in grasses with P_{CO2}. Different symbols and colours indicate the three different harvest intensities and the three classes of P fertilization, respectively. Trend lines were fitted as exponential rise to maximum (3 parameter, $y=y0+a\times(1-e^{(-b\times x)})$) for V and W+B. Significance levels are: * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$.

4.3.4. Identification of soil P thresholds

Because we observed stronger coefficients of determination for the two groups V and W+B for P_{CO2} we derived the soil P threshold for the two groups separately. For P_{AAE} the combined dataset was used. Nevertheless, similar to chapter three the three plant indicators partly resulted in different thresholds. We observed higher values for P_{CO2} and P_{AAE} at the low threshold related to relative yield than to grass P concentration and PNI. The derived thresholds are shown in Table 4.2.

Table 4.2: Mean and 95% confidence interval of P_{CO2} and P_{AAE} as related to four levels of relative yield, P concentration and PNI. The relationship is reflected by the function $y = y0+a*(1-e^{(-b*x)})$, y = plant indicators and x = soil P extract. For the maximum of the function no 95% confidence range is shown. The value at which maximum is reached is shown in brackets. For the synthesis of the threshold derived by the three plant parameters the highest observed value was selected excluding aor.

Plant indicator	**P_{CO2} (V)**		**P_{CO2} (W+B)**		**P_{AAE}**	
			mg kg^{-1}			
Level	**Mean**	**95% confidence interval**	**Mean**	**95% confidence interval**	**Mean**	**95% confidence interval**
Relative yield						
90%	0.26	0.21 -0.32	0.6	0.5 -0.75	8.3	5.8 –aor
95%	0.30	0.25 -0.44	0.75	0.62 -0.96	11.8	7.6 –aor
100%	0.40	0.3 -aor	1.0	0.8 -aor	aor	10.6 -aor
maximum	0.7 (105%)		1.5 (102%)		20.3 (96.5%)	
P concentration						
2.1	0.20	aor -0.24	0.45	0.4 -0.5	6.3	5.4 -7.3
3	0.32	0.27 -0.47	0.8	0.7 -0.95	12.2	10.2 -14.0
3.5	0.56	0.38 -aor	1.2	1.0 -1.4	18.6	15.6 -23.0
maximum	>0.67 (>3.6)		3.1 (4.2)		40 (3.9)	
PNI						
80	0.22	aor -0.26	0.45	0.38 -0.52	6.5	5.7 -7.5
100	0.30	0.25 -0.36	0.7	0.58 -0.83	10.4	9.1 -12.2
120	0.45	0.34 –aor	1.4	1.1 –aor	22.0	17.2 -aor
maximum	>0.6 (>127)		2.8 (126)		33 (123)	
Thresholds						
Low	**0.26**	0.21 -0.32	**0.6**	0.5 -0.75	**8**	5.8 -7.5
Middle	**0.32**	0.27 -0.47	**0.8**	0.7 -0.96	**12**	10.2 -14
High	**0.56**	0.38 -aor	**1.4**	1.1 -1.4	**22**	17.2 -23.0

aor = out of range

4.4. Discussion

4.4.1. Soil P status classes

Although P_{CO2} and P_{AAE} generally reflected the differences between P fertilizer treatments, the two methods sometimes resulted in a different classification of the soil P status (Table 4.1). This observation is in line with reports by Stünzi (2006) and Flisch et al. (2009). The results shown in this chapter are for the 0 to 5 cm soil layer, but the routine soil tests are conducted on soil samples of the 0 to 10 cm soil layer. We observed always lower values in the 5 to 10 cm soil layer than in the 0 to 5 cm layer (Table A.9). This indicates a dilution of P_{CO2} and P_{AAE} when the 0 to 10 cm layer is considered. Thus, we consider the discussion of the data of the soil layer 0 to 5 cm principally applicable to the 0 to 10 cm soil layer.

In V, yield and plant P nutrition status were lowest in N1P0K0 allthough it had a similar soil P class for P_{CO2} as N1P1K1. Additionally, yield and PNI were not significantly higher in N1P2K2 than in N1P1K1, but the related soil P status classes for P_{CO2} were identified as B and C, respectively, according to Flisch et al. (2009). This indicates a potential underestimation of the soil P status for both treatments, because N1P1K1 was fertilized according to average P uptake (Table 2.3) and N1P2K2 overfertilized. The low P nutrition status classes in V determined by P_{CO2} could partly be explained by the high clay content as stated in Flisch et al. (2009). However, the clay content is considered for the derivation of the soil P classes. Additionally, the plant indicators show that P was sufficiently available to the plants. The high P buffer capacity of the soil in V could explain this observation. The high buffer capacity for P in V was reflected by the isotope exchange parameters r_1/R and n, which ranged from 0.19 to 0.33 and 0.42 to 0.52, respectively (Table A.7). Oxalate extracted iron (Fe_{ox}) of about 6500 mg Fe kg^{-1} and aluminum (Al_{ox}) of about 1500 mg Al kg^{-1} in relation to oxalate extracted P (P_{ox}) between 242 to 384 mg P kg^{-1} also indicates a high P buffer capacity (Table A.8). Both methods have been suggested to improve evaluation of soil P availability compared to selected soil routine tests, especially on soils with high P sorption capacity (Bell et al., 2005b; Morel et al., 2000).

That all treatments in W were classified as poor in P (class A) by P_{AAE} strongly contrasts with the obtained yields and the plant P nutrition status identified by PNI for the P fertilized treatments. Thus, in W the soil P status classification is not appropriate.

P_{AAE} was reported to be affected by pH (Demaria et al., 2005) and calcium and carbonate content (Stünzi, 2006). That an increasing pH reduces P_{AAE} (Demaria et al., 2005) might explain the lower P_{AAE} found in N1P1K2org in W compared to the mineral P1 treatments in W, because the pH was significantly higher in W-N1P1K2org (Table 2.2). However, the mean pH in W (5.9) was similar to V (5.8). Additionally, P exchangeable in one minute (E_{1min}) of the mineral P fertilized treatments in V and W (Table 3.2) were in a similar range, indicating a sufficient P availability in the soil. This also indicates that P_{AAE} underestimated the P availability in W.

Nevertheless, P_{AAE} in the P unfertilized and P fertilized treatments were significantly different in W. Thus, a modification of the interpretation scheme could improve the interpretation of P_{AAE} for soil P status classes.

4.4.2. Thresholds derived for P_{CO2} and P_{AAE}

The procedure to derive the three thresholds low, middle and high (Table 4.2) was described in chapter three (section 3.3.6.). There the applicability of the exponential function and the combined use of relative yield, grass P concentration and PNI were discussed in detail. For P_{CO2} we defined the soil P thresholds separately for V and W+B because the soil in V had a much higher P sorption capacity as revealed by isotope exchange parameters (Table A.7) and higher Fe_{ox}+Al_{ox} values (Table A.8). This seems justified because the fitted exponential functions showed higher coefficients of determination for the two soil groups (V: $r^2 = 0.53$ and W+B: $r^2 = 0.69$) than for the overall function ($r^2 = 0.25$).

The thresholds low and high, which frame the range of sufficient P availability (Figure 3.1) were close to the threshold of 0.5 mg P kg^{-1} reported for crops by Gallet et al. (2001). For V, the threshold of 0.5 mg P kg^{-1} was similar to the high threshold, indicating that no decreases in yield may be expected above that value. For W and B this value is close to the low threshold. The application of a high threshold of 1.4 mg P kg^{-1} would be appropriate for W+B.

For P_{AAE} the combination of all treatments from the three locations seems justified for the derivation of soil P thresholds. For “low” and “high” we found 8 and 22 mg P kg^{-1}, respectively. This is much lower than the 35 mg P kg^{-1} reported by Gallet et al. (2001) for crops. But their value was between the two values of 40 and 33 mg P kg^{-1} found as

the maximum of the curves for grass P concentration and PNI in our study. The small changes in grass P concentration and PNI from high to maximum (Table 4.2) were related to relatively high changes in P_{AAE} without yield response. Thus, we consider the application of the high threshold as appropriate to identify surplus P nutrition status. This is in contrast to the interpretation for soil P status classes proposed by Flisch et al. (2009). The P_{AAE} values of 22 and 35 mg P kg^{-1} from our study and from Gallet et al. (2001) would result in soil P classes of A and B for most soil types. This suggests that the interpretation scheme is too high.

In conclusion, we found close relationships between plant indicators and P_{CO2} and P_{AAE} analyzed in the 0-5 cm soil layer. For P_{CO2} we propose two separate thresholds for the soil in V and the soils in W+B, respectively. The low and high thresholds are 0.26 and 0.56 mg P kg^{-1} for V and 0.6 and 1.4 mg P kg^{-1} for the soils W+B. For P_{AAE} the interpretation without additional soil information was reasonable for the investigated locations. We propose 8 and 22 as low and high threshold, respectively. For both, P_{CO2} and P_{AAE} the soil P availability in the 0 to 5 cm soil layer was underestimated by the soil P classification scheme. Plant P indicators and parameters estimating the P buffer capacity of soils improved the estimation of P availability and can be used to adjust or assist the soil routine tests.

Chapter 5: Seasonal dynamics of microbial phosphorus in a permanent grassland

5.1. Abstract

The effect of different P fertilizers on the turnover of phosphorus (P) through the microbial biomass and its consequences for P availability in soils are not well understood. This is particularly true for grassland soils where organic matter is accumulated in the topsoil, microbial activity is high and plant cover changes during the season. The amounts of available inorganic P and microbial P can fluctuate over the season, but the drivers controlling these variations are not well known. We studied three P input treatments: no P (P0), mineral P (Pmin) and organic P (Porg) fertilization, with 17 kg P ha^{-1} yr^{-1} applied as superphosphate and slurry, respectively, in permanent grassland with three harvests per season. Throughout the growing season of 2009 (241days), soils (0-5 cm) were sampled every 2-3 weeks and plant biomass at each harvest. Available P was measured as resin-extractable P (P_{res}). Microbial P (P_{mic}) was determined by hexanol fumigation with simultaneous extraction by anion exchange resin membranes, without application of correction factors. From the observed fluctuation in P_{mic} we calculated P_{mic} turnover and fluxes. The lowest yield and plant P uptake were observed in P0 and the highest in Pmin. During the season, P_{res} ranged from 0.9-3.5, 5.3-11.2 and 1.9-6.7 mg kg^{-1} $soil^{-1}$ and P_{mic} from 20-44, 43-59 and 61-93 mg kg^{-1} $soil^{-1}$ in P0, Pmin and Porg, respectively. Consistent increases of P_{mic} in the three treatments were observed after the summer and autumn harvests when plant growth rate was low. Reduced P_{mic} and increased P_{res} were observed after dry periods. The turnover rate of P in the microbial biomass tended to be higher in P0 than in Pmin and Porg. The fluxes of P through the microbial biomass and the mean stocks of P in the microbial biomass were two to three times higher than P uptake in harvested aboveground biomass during the season 2009 (241 days). The fluctuations in P_{res} and P_{mic} are discussed in relation to climate, sward management and plant growth.

5.2. Introduction

Efficient P management in grassland requires exact knowledge of P availability and dynamics to avoid yield decreases by insufficient P fertilization and P accumulation in the soil by surplus P fertilization. Available P interacts with the soil microbial biomass through microbial P (P_{mic}) immobilization, organic P mineralization and P release (Oberson and Joner, 2005), and with plants through plant P uptake. Contents and turnover of P_{mic} are generally high in grassland soils (Blackwell et al., 2010). Both available P and P_{mic} are affected by sward management such as fertilizer input and cutting regime. When similar amounts of P are applied, long term inputs of mineral fertilizers often lead to a better P availability than inputs of organic fertilizers (Oberson and Frossard, 2005). At the same time, organic fertilized systems have higher P_{mic} and biological activity (Oberson et al., 1996; Oehl et al., 2001b) and thus P_{mic} may buffer P availability to plants (Macklon et al., 1997). However, plants and microbes both draw from the available P pool in the soil and competition for P uptake may occur (Reynolds et al., 2003). On P deficient soils P cycling can be increased (Magid et al., 1996). However, it is not well understood if plants profit from the increased P turnover in the microbes or if competition for P prevails. Both the pool size of P_{mic} and the rate of P turnover determine the rate of P cycling and the availability of P to plants.

The main factors affecting microbial growth and thus its pool size are substrate and nutrient input, temperature and soil moisture (Dalal, 1998). The main belowground carbon inputs in grassland soils are root turnover and exudation, while aboveground inputs include litter fall and organic fertilization. Besides carbon, other nutrients might influence microbial biomass as well. Particularly for P in grasslands, a shortage in P availability induced by plant growth could affect microbial growth directly or indirectly by reduced carbon flows from the roots (Van Veen et al., 1989). Temperature and soil moisture are determined by the geographical location and thus the prevailing climatic conditions. However, relationships of P_{mic} to temperature and soil moisture have mostly been studied in incubation experiments (Grisi et al., 1998; Joergensen et al., 1990; Jupp and Newman, 1987). Freezing-thawing and drying-rewetting have been identified as major factors affecting available P and P_{mic} (Blackwell et al., 2010). Little is known about these relationships under field conditions and how they affect P availability and plant P uptake.

Regardless of the general P nutrition status of grasslands the above described factors cause fluctuation during the season (Tate et al., 1991). In temperate grasslands increases of P_{mic} in winter have been observed in most studies (Chen et al., 2003; Perrott et al., 1990; Tate et al., 1991). Perrott et al. (1990) concluded that the main P release from the microbial biomass during the year occurred in spring. Such seasonal fluctuations of P_{mic} have been used to estimate P fluxes and release (Brookes et al., 1984; Oberson et al., 1996) and P turnover by the microbial biomass (McGill et al., 1986; v. Lützow and Ottow, 1994). The greater fluctuation of P_{mic} in grasslands compared to arable land results also in greater P turnover, and P fluxes often exceed plant P demand (Blackwell et al., 2010). To improve the information about plant availability of P, the simultaneous measurement of available and microbial P pools was proposed by He et al. (1997). However, the interaction of P_{mic} with available P and the controlling factors and processes are not well understood.

In this study we investigated effects of P fertilization on the seasonal dynamic of P_{res} and P_{mic} under field conditions. We hypothesized that the seasonal dynamics of P_{mic}, its turnover and the fluxes through P_{mic} are affected by the type of P fertilization. We further assumed that P_{mic} interacts with P_{res}. and that this interaction is stronger under organic fertilization. In addition, we wanted to assess effects of plant growth and climatic conditions on the seasonal dynamics of P_{res} and P_{mic}. We hypothesized that the plant growth rate, sward cutting and changes in weather conditions are reflected by changes in P_{res} and P_{mic}. For this study we investigated a species-rich grassland harvested three times per year. Three types of P fertilization (no P (P0), mineral P (Pmin) and organic P (Porg)) were selected from a fertilizer trial established in 1992. Soils and plants were sampled regularly and soil and weather conditions monitored during the season in 2009. We analyzed available and microbial P on field moist soil samples and measured plant dry matter production and P uptake.

5.3. Material and Methods

5.3.1. Experimental site, management and treatments

The fertilizer experiment is located near Watt (W) in the canton of Zürich (47°26'44"N, 8°29'32"E, at 500 m above sea level) and managed by the Federal Research Station Agroscope Reckenholz-Tänikon (ART) since 1992. Average annual temperature and precipitation between 1999 and 2009 were 9.8°C and 1077 mm. The mesic grassland is well established on a medium deep (50-70 cm) Cambisol with medium base saturation (50-80% of the cation exchange capacity are basic cations). The average soil texture in the top 10 cm of the soil measured at the beginning of the experiment was 220, 340 and 440 g kg^{-1} soil of clay, silt and sand, respectively (Phillip et al., 2004). The vegetation is an *Arrhenaterion elatioris* association with 30-40 angiosperm species. It is harvested three times per year. For conservation purposes the first harvest takes place after June 15. The late cut of the herbage leads to lower forage quality of that particular harvest caused by the advanced growth stages of the plants. In 2009, harvests took place on day 167, 216 and 267.

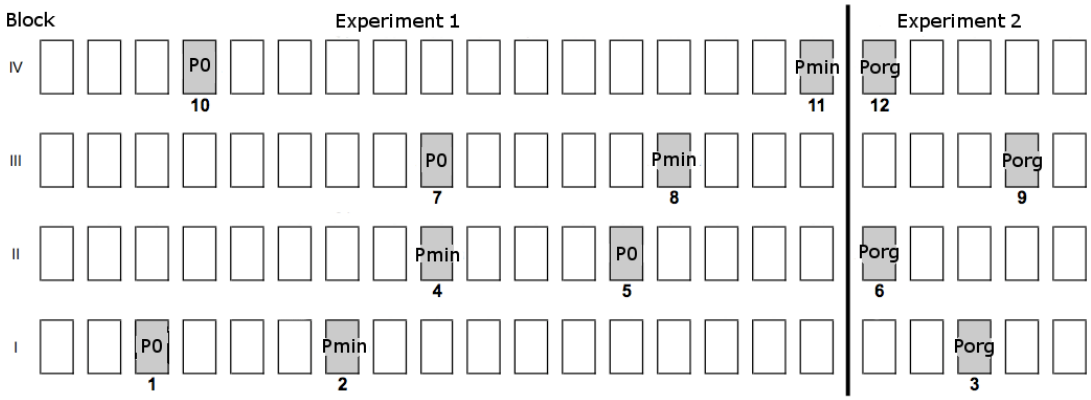

Figure 5.1: Experimental set up of the investigated fertilizer treatments P0, Pmin and Porg in the long term fertilizer trials in Watt in the mesic grassland managed at low intensity since 1992.

In total three fertilizer treatments were chosen. Two mineral fertilized treatments were from the same experiment, fertilized either with N and K (P0) or with N, P and K (Pmin). In addition, an organically fertilized treatment (Porg) was selected from a neighbouring experiment located on the same site (Figure 5.1). Both experiments had a randomized block design with a plot size of 2 m x 5 m. For mineral N, P and K

fertilization, fertilizers were applied as ammonium nitrate (split into three applications: in spring, after the first and after the second cut), super-phosphate and potash (applied in spring). The additional treatment was organically fertilized with annual application of 24 m^3 ha^{-1} dairy slurry, split into a spring application at the beginning of pasture growth and another application after the first harvest (Figure 5.2). For spreading the slurry was diluted one to one with tap water. Amounts of applied mineral nutrients and the estimated nutrient application of the organic fertilization are shown in Table 5.1.

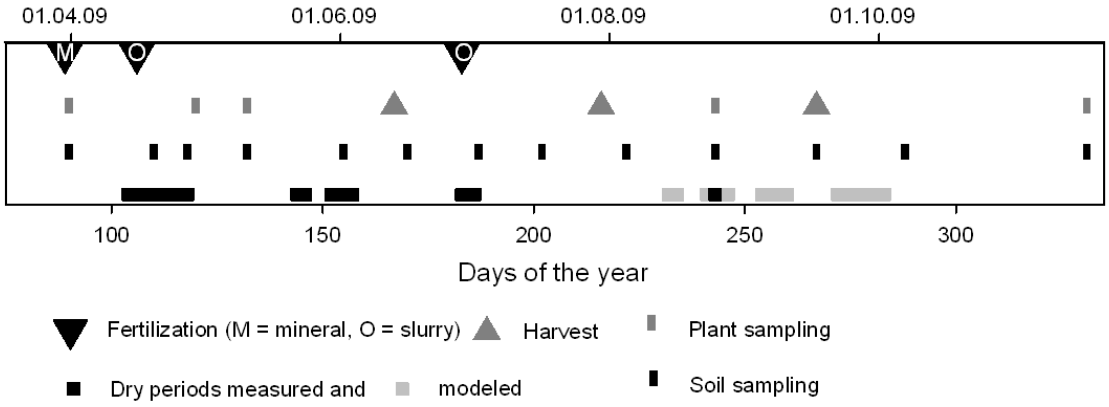

Figure 5.2: Overview of experimental management and sampling dates in 2009

Table 5.1: Amounts of fertilizer N, P and K applied annually since 1993 in the three treatments P0, Pmin and Porg in a mesic grassland managed at low intensity.

Treatment	Fertilizer	N	P	K
			kg $ha^{-1}yr^{-1}$	
P0	mineral	45	0	83
Pmin	mineral	45	17	83
Porg	dairy slurry	55[#]	17[#]	102[#]

[#] Estimated assuming 1:1 dilution of dairy slurry and water according to Flisch et al. (2009).

5.3.2. Soil sampling

Soils were sampled 13 times throughout the season from March to November 2009 (Figure 5.2). The sampled period covered a total of 241 days. Sampling dates were chosen to reflect the effect of fertilization, harvests and environmental conditions such

as dry spells and precipitation events. At each sampling, 3 randomly distributed soil cores of 2 cm diameter and 5 cm length were taken from each plot, excluding the outer 0.5 m of the plot. The three cores were pooled into one composite sample, which was stored at 4°C in the dark. Within two days samples were sieved through a 2 mm mesh, cleaned from visible plant material and gravimetric water content was measured. P_{res} and P_{mic} were measured on these samples not later than four days after sampling. Remaining soil was air dried and used for determination of general soil properties as shown in Table 5.2. Soil organic C and total N were significantly higher in Porg than in P0 and Pmin. Total P and pH were ascending from Porg to P0 and total organic P was lower in P0 than in Porg and Pmin.

Table 5.2: Means and standard deviations of selected soil properties of the three fertilizer treatments P0, Pmin and Porg in the top 5 cm of the soil under a mesic grassland managed at low intensity. Different letters indicate significant differences (LSD test at alpha = 0.05, with Holm adjusted p-values).

Treatment	Total C[a]	Total N[a]	Total P[b]	Total organic P[c]	pH[d]
	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	(in H_2O)
P0	24.7 ±0.5 b	3.0 ±0.1 b	664 ±3.1 c	366 ±26.0 b	5.2 ±0.1 c
Pmin	24.9 ±1.2 b	3.1 ±0.2 b	784 ±12.7 b	439 ±21.0 a	5.4 ±0.1 b
Porg	29.8 ±1.0 a	3.8 ±0.1 a	834 ±6.4 a	480 ±5.2 a	6.8 ±0.1 a

[a] measured by CN analyser
[b] extraction with 0.5 M H_2SO_4 after incineration
[c] according Saunders and Williams (1955)
[d] Soil:H_2O ratio = 1:2.5

5.3.3. Plant sampling and analysis

Plant sampling took place one to two days prior to each harvest in the field (Figure 5.2). In Pmin plants were sampled on five additional dates. Plants were cut at four cm above the ground using electric scissors on a randomly selected area of 50 cm x 50 cm with at least 50 cm distance to the plot border. In order not to affect the seasonal productivity pattern of the sward we avoided double sampling on the same spot. Therefore, the number of plant samplings was restricted because of the small plot size. The plant samples were dried at 60°C for at least three days before weighing for dry matter determination. Dry matter was used to calculate the total yield and the growth rate of the Pmin swards. The daily growth rate was calculated as the difference of dry matter

between two following plant samples (t2-t1) divided by the number of days between the sampling dates.

Subsamples of the plant material were milled, ignited (550°C for 8h) and extracted in concentrated HNO_3. The P concentration was measured colorimetrically with the malachite green method (Ohno and Zibilske, 1991).

5.3.4. Microbial and available phosphorus

Resin-extractable P (P_{res}) and microbial P (P_{mic}) were determined after Kouno et al. (1995) by extraction with anion exchange resin membranes with simultaneous hexanol fumigation for P_{mic}. For extraction 3 g field moist soil were shaken with 30 ml of double-distilled water and two resin strips of 3 x 2 cm (BDH Laboratory Supplies #55164 2S, Poole, England) for 16 h at 150 rpm on a horizontal shaker. After rinsing the membranes with double-distilled water, P was eluted by shaking for 2 h in 0.1 M NaCl/HCl solution. The P concentrations in the eluates from unfumigated (P_{res}) and fumigated (P_{fum}) subsamples were measured colorimetrically as described above. For each soil sample the extraction was done in duplicates and averaged. To calculate P_{mic}, mean P_{res} was subtracted from mean P_{fum}. The P sorption of the soil was tested on a similar set of soil samples taken prior to this experiment by addition of 20 µg P to each sample. The mean recovery ± standard deviation of the P addition was 93 ± 43 %. Because of the high recovery and its high variation we did not correct for P sorption. We did not use a conversion factor (k_p) to calculate P_{mic} because we did not determine it for our soils. The k_p can be highly variable for different soils and microbial communities (McLaughlin et al., 1986; Oberson and Joner, 2005). During the seasonal measurements a control soil (unfertilized and dried soil from the same site) was always included. Mean P_{res} and P_{mic} ± standard deviation of the control soil were 10.1 ± 0.5 and 7.9 ± 0.9 mg kg^{-1}, respectively. Because of the low variation between measurements we used no correction to account for analytical variation between extraction dates.

5.3.5. Calculation of P fluxes and microbial P turnover

The P_{mic} turnover rate (equation 1) was estimated according to von Lützow and Ottow (1994). They applied the concept of McGill et al. (1986) to estimate microbial carbon turnover. The principle of the calculation is that the sum of P_{mic} losses ($\sum P_{mic}$ losses =

the sum of P_{mic} decreases between two subsequent measurements) during the season is turned over by the mean seasonal microbial biomass:

$$P_{mic}turnover\ rate = \frac{\sum P_{mic} losses}{meanP_{mic}} \quad [1]$$

The $\sum P_{mic}$ losses and mean P_{mic} are given in mg kg^{-1} dry soil and the P_{mic} turnover rate is calculated for the period of 241 days of this experiment. Dividing the 241 days of the investigated season by the P_{mic} turnover rate results in the mean P_{mic} turnover time in days. The P flux was then derived as shown in equation 2:

$$Pflux = \frac{meanP_{mic} * \rho * D}{P_{mic}turnover\ rate} \quad [2]$$

where ρ is the bulk density of the soil of 1.05 g cm^{-3} and D is the sampling depth of 5 cm. The resulting Pflux is given in kg P_{mic} ha^{-1} during a period of 241days.

5.3.6. Climatic data

On site a meteorological station was installed (DATAHOG2, Skye Instruments ltd, Powys, UK) that measured soil temperature (T_{bg}) at 5 cm below the soil surface (Figure 5.3) and air temperature (T_{air}) at 200 cm above it. A data logger (HOBO Micro Station, Onset Computer Corporation, Bourne, US) was implemented to measure soil moisture (sensor: S-SMC-M005) at 5 cm depth. Due to technical problems, soil moisture, T_{bg} and T_{air} were not recorded from July 19 to August 6 and again after August 13, 2009. The additional environmental data such as precipitation, canopy temperature at 5 cm above the ground (T_{ag}) as shown in Figure 5.3 and global radiation (G_R in W m^{-2}) were obtained from the closest meteorological station (Zürich Affoltern, 47°26'N, 8°31'E, 443 m above sea level, direct distance to the field ≈ 2 km) available from MeteoSwiss, the Federal Office of Meteorology and Climatology (IDAWEB, 2009). Since T_{air} and precipitation measured in the field and at the official meteorological station were similar we used the complete data set from the Meteo station for T_{air}, precipitation and radiation. For periods without field data the soil moisture was modelled using a simple water balance approach (equation 3) on a daily base.

$$0 = \Pr ecipitation + ETPkc + R + \Delta W \quad [3]$$

The ETP_{kc} is the evapotranspiration corrected for plant growth, R is the water loss from the soil, e.g. by percolation or runoff, and ΔW is the change in soil water content. The

ETP was calculated according to equation 4 derived from Turc (1961) and corrected for plant size using a crop coefficient k_c in the next step (equation 5).

$$ETP = 0.027 * (G_R + 24) * \frac{T_{ag}}{T_{ag} + 15}, \quad [4]$$

$$ETPkc = kc * ETP \quad [5]$$

The coefficient k_c ranged from 1.0 in the young or freshly cut sward to 1.5 in the mature sward as adapted from the DVWK bulletin (1996). To estimate R we used the difference between precipitation and the change of soil moisture measured in the field in the first half of 2009. The water input from precipitation was lost from the 0 to 5 cm soil layer when precipitation exceeded 5 mm. Thus, R is the difference between actual precipitation (when > 5mm) and 5. Below 5 R is zero. When precipitation was below 0.4 mm soil moisture was not affected. Thus, only a precipitation between 0.4 to a maximum of 5 mm was considered for the calculation of soil moisture. The ΔW of the soil was estimated applying the linear relationship of soil moisture decrease and calculated ETP_{kc} during periods without precipitation. Five periods of five to nine days with soil moistures between 100 and 250 g H_2O kg^{-1} soil were identified. On average, 264 g kg^{-1} (r^2=0.99) of ETP_{kc} was resupplied from the soil (data not shown), meaning that one quarter of the evaporated water came from the 0 to 5 cm soil layer. Thus, the change in water content (ΔW) was calculated as 0.264*ETP_{kc}. To minimize errors, the model was readjusted at each sampling date to the measured gravimetric water content. Both measured and modelled soil moistures are shown in Figure 5.4.

5.3.7. Statistical analysis

Statistical analyses were performed with R version 2.8.1 (R Development Core Team, 2008) using the library agricolae v. 1.09 (Mendiburu de, 2009) .Because the treatments were located in two different experimental designs, fertilizer treatments were compared only with a F-test. If the F-test was significant at $p<0.01$, a test for least significant difference (LSD) with an alpha of 0.05 was conducted. The Holm adjustment for p-values was always applied to control for false positives in multiple comparisons. Prior to analysis percentage data of soil moisture were transformed to their arc sin. Correlation coefficients (ρ) and their level of significance were calculated by the

Pearson product-moment correlation. Significance levels are given as ns. = not significant, * = $p<0.05$, ** = $p<0.01$ and *** $p<0.001$.

5.4. Results

5.4.1. Climate conditions

A typical temperature curve for temperate climates with a cold winter and a warm summer was observed (Figure 5.3). We recorded a total of 246 vegetation days (T_{ag}> 5°C) in 2009, with a continuous period between day 85 and 287. Maximum T_{ag} was recorded in July and August, with monthly mean temperatures of 19 and 20°C, respectively. Soil temperature (T_{bg}) generally followed the trend of T_{ag}, lagging behind slightly (Figure 5.3). High T_{bg} of above 20°C was observed between day 179-188 and 218-243. Maximal T_{bg} was reached on day 183 and 231 with 24.4 and 24.8°C, respectively.

Precipitation (Figure 5.3) was reasonably well distributed throughout the season with a monthly average of 85 mm (± 46). April was exceptionally dry with 15 mm (day 71-116). The soil moisture measured with the sensor in the field was in accordance with the soil moisture measured gravimetrically in the lab and mainly reflected the distribution of precipitation (Figure 5.4). Soil moisture below 200 g kg^{-1} was measured only on day 116, 156 and 246. Soil moisture was below 300 g kg^{-1} for at least three consecutive days during eight periods as shown in Figure 5.2. These periods are hereafter referred to as dry periods.

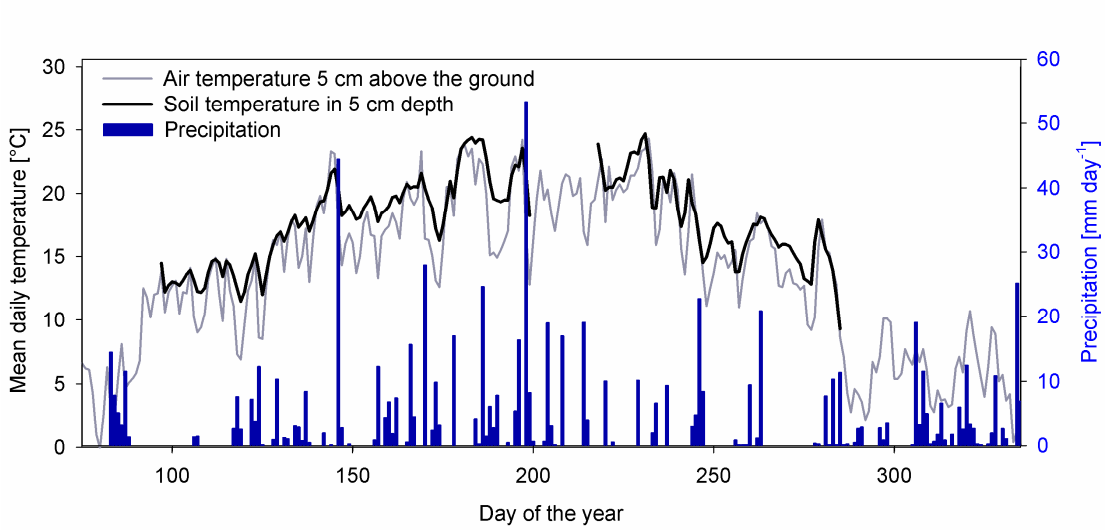

Figure 5.3: Daily precipitation (bars), canopy temperature (5 cm above soil, grey line) and soil temperature (top 5 cm of the soil, black line) during the season 2009. Precipitation and aboveground temperature were measured by a meteorological station in Zürich-Affoltern and soil temperature at the investigated field site.

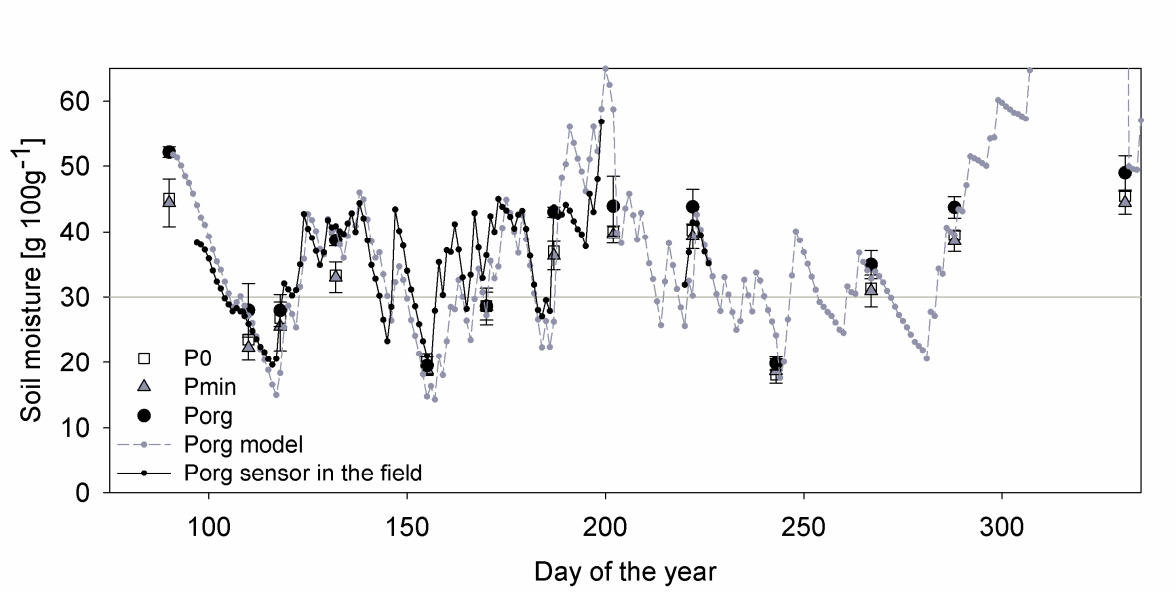

Figure 5.4: Soil moisture during the season 2009 as measured in the field (black dots), modeled (grey dots) in Porg and gravimetrically measured in the lab for P0, Pmin and Porg (symbols). Error bars indicate standard deviation. The solid grey line indicates a soil moisture of 300 g kg^{-1}.

5.4.2. Plant growth

Plant growth rates measured in Pmin were highest between day 120-132 and 218-243 and lowest at the beginning and end of the season (Figure 5.5). Total harvested plant dry matter was significantly different between treatments, with the highest yield achieved in Pmin and the lowest in P0 (Figure 5.6). Yield differences were most pronounced at the first harvest. Total plant P uptake (Figure 5.6) reflected similar

treatment differences as the biomass. In contrast to dry matter of the individual harvests, P uptake differed significantly between treatments at each of the three harvests. In Pmin the P uptake was always higher than in P0 whereas it was intermediate in Porg.

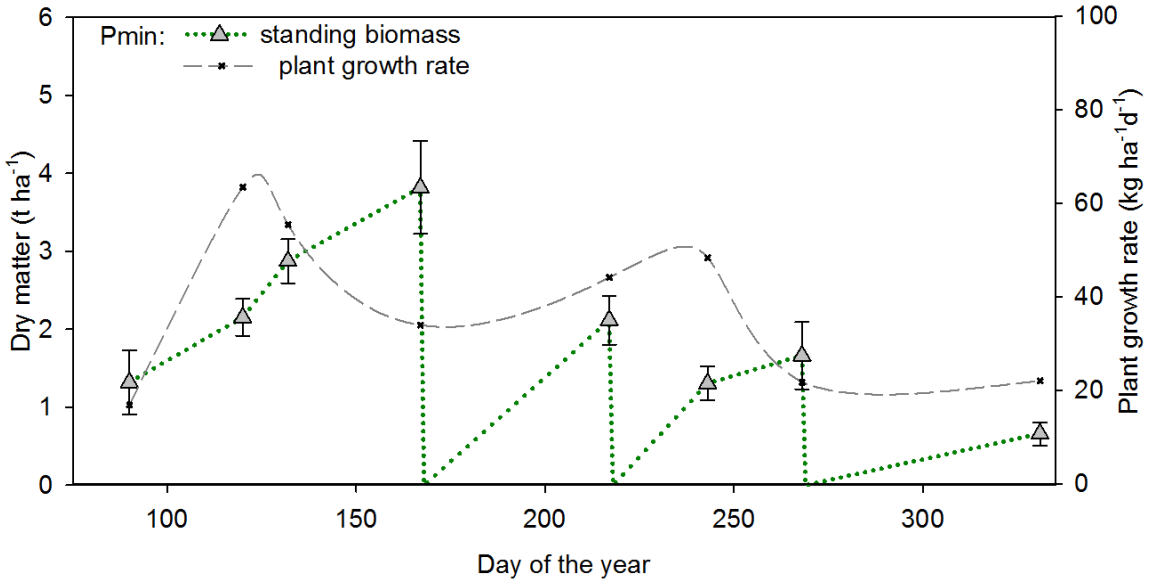

Figure 5.5: Aboveground plant dry matter (t ha^{-1}) and growth rate (kg ha^{-1} d^{-1}) during the season in the Pmin treatment in the mesic grassland managed at low intensity in Watt 2009. Error bars indicate standard deviation.

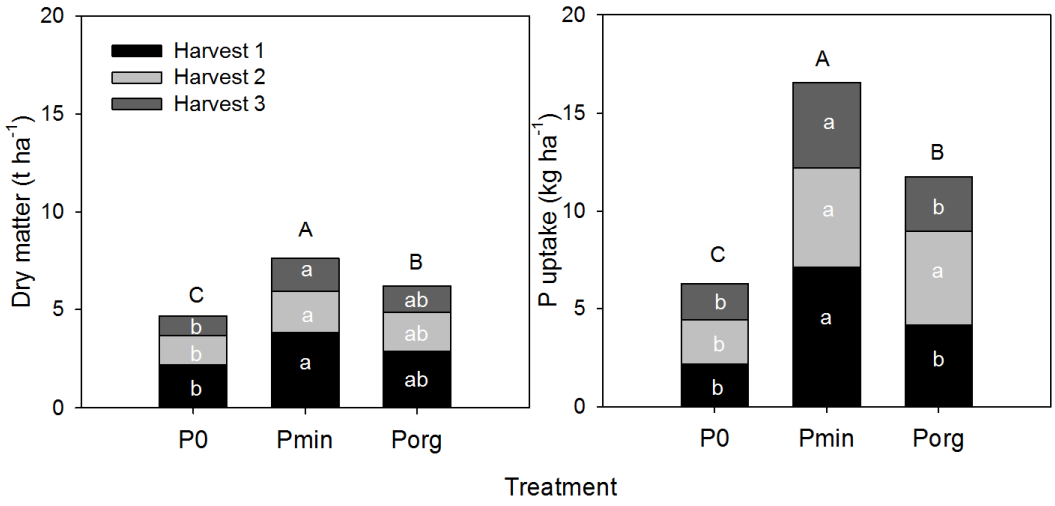

Figure 5.6: Total aboveground plant dry matter (t ha^{-1}, left) and P uptake (kg ha^{-1}, right) as affected by harvest and P fertilization. Different letters indicate significant differences in total P uptake between treatments (capitals) and harvests (lower case) according to a LSD test (alpha = 0.05, with Holm adjusted p values).

5.4.3 Resin extractable and microbial phosphorus

Mean P_{res} was highest in Pmin, and the same was also observed at most of the individual sampling dates (Figure 5.7). Over the entire season, P_{res} fluctuated from 0.3 to 3.5, 5.4 to 12.7 and 1.9 to 6.5 mg P kg^{-1} for P0, Pmin and Porg, respectively. The seasonal pattern of P_{res}-fluctuation (Figure 5.7) was similar for all treatments. However, the amplitude of P_{res} was larger in Porg than in P0 and Pmin. Maxima of P_{res} were observed at day 110, 155, 187 and 243 and coincided with dry periods shortly before or at this sampling date. In all three treatments, P_{res} was exceptionally high at day 187, coinciding with low plant growth rates, high T_{ag} and T_{bg} and a slurry application in Porg shortly before. The negative relationship of P_{res} and soil moisture was most pronounced in Pmin (Figure 5.8).

In contrast to P_{res}, highest P_{mic} was found in Porg whereas P_{mic} in P0 was again lowest (Figure 5.7). Over the entire season, P_{mic} fluctuated from 19.5 to 44.1, 42.7 to 58.8 and 53.3 to 93.5 mg kg^{-1} for P0, Pmin and Porg, respectively. Although fluctuations in microbial P were observed in each of the three treatments, their patterns and amplitudes differed significantly between treatments. A simultaneous increasing trend of P_{mic} for the three treatments was only observed after days 155 and 267, each time following a harvest at a time of low plant growth. Minima of P_{mic} were observed on day 110-118 and 155 in Porg and on day 187 in P0. A positive relationship of P_{mic} with soil moisture was observed (Figure 5.8).

The sum of P_{mic} losses showed the same treatment differences as mean P_{mic} (Table 5.3) with the highest in Porg and lowest in P0. The P_{mic} losses in Pmin and Porg were similar to the P_{mic} pool size whereas in P0 losses were higher than P_{mic}. The P_{mic} turnover rate ranging from 1 to 1.4 was similar between treatments. The P_{mic} flux was lowest in P0 with 24.1 and highest in Porg with 38.1 kg P ha^{-1} $season^{-1}$. For P_{mic} stocks the picture was similar with lowest values in P0 (17.5 kg P ha^{-1}) and highest in Porg (36.6 kg P ha^{-1}). Both were higher than the actual plant P uptake in the three treatments.

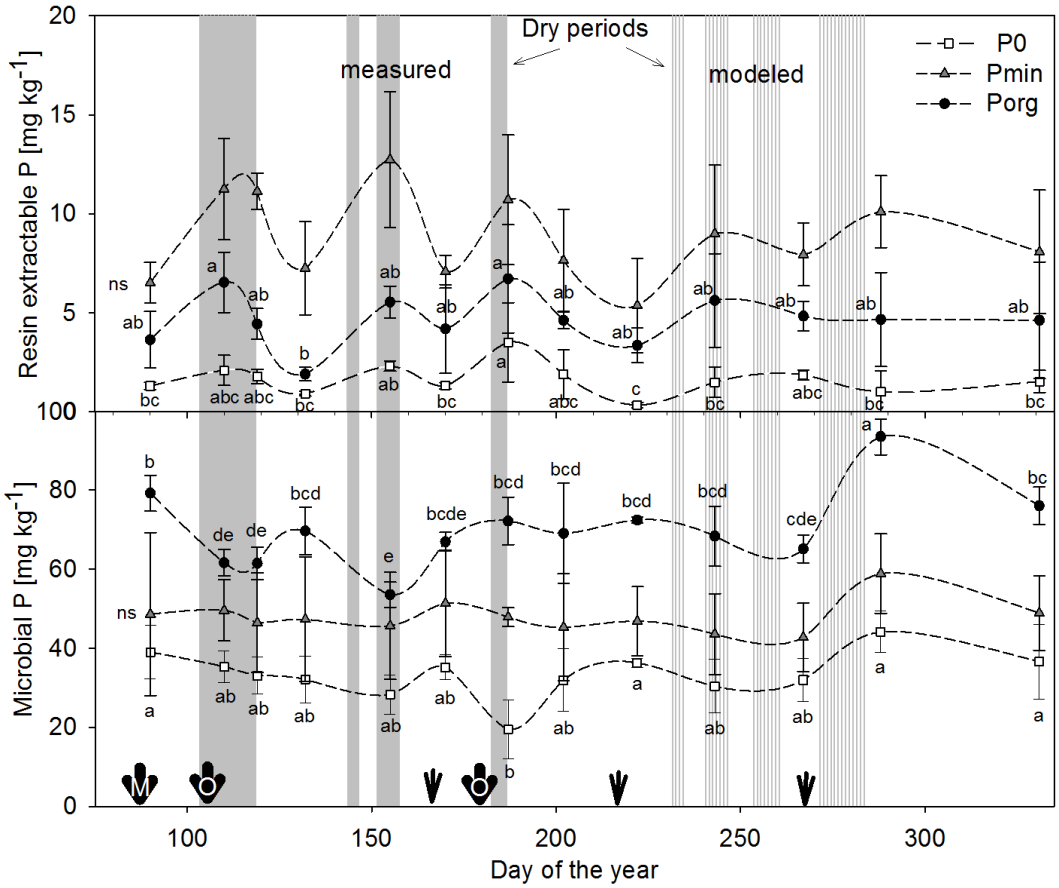

Figure 5.7: Fluctuation of resin-extractable and microbial P in the three fertilizer treatments P0, Pmin and Porg in a mesic grassland managed at low intensity during the growing season in 2009. Error bars indicate standard deviation of four replicates. Different letters indicate significant differences between sampling points analysed separately for each treatment (LSD test at alpha = 0.05, with Holm adjusted p-values). Grey and grey striped bars indicate measured and modelled days with gravimetric water content in the field below 30 g 100 g^{-1} for at least three days in a row. Arrows at the bottom x-axis indicate fertilizer application (bold, M=mineral, O=organic) and harvest (slim).

Table 5.3: Mean resin-extractable and microbial P pool and calculated P fluxes and turnover in the top 5 cm of the soil in Watt as affected by P fertilization. Mean values are followed by ± standard deviation. Least significant differences (LSD) calculated for alpha = 0.05, with Holm adjusted p-values.

Parameters	P0		Pmin		Porg		LSD
Mean P_{res} (mg kg^{-1})	1.6	±0.6	8.9	±2.3	4.7	±1.5	2.0
Mean P_{mic} (mg kg^{-1})	33.5	±5.5	48.0	±11.1	70.2	±4.7	10.2
∑ of P_{mic} losses (mg kg^{-1})	45.9	±11.2	53.1	±11.1	72.5	±15.6	26.5
P_{mic} turnover rate ($season^{-1}$)	1.4	±0.4	1.1	±0.3	1.0	±0.2	0.6
P_{mic} turnover time ($days^{-1}$)	183	±47	222	±59	242	±54	110
P_{mic} flux (kg ha^{-1} $season^{-1}$)	24.1	±5.9	27.9	±5.8	38.1	±8.2	13.9
Mean P_{mic} stock (kg ha^{-1})	17.5	±2.0	24.9	±4.9	36.6	±0.9	6.4
Total plant P uptake (kg ha^{-1} yr^{-1})	6.2	±1.1	16.6	±1.4	11.7	±1.5	2.7

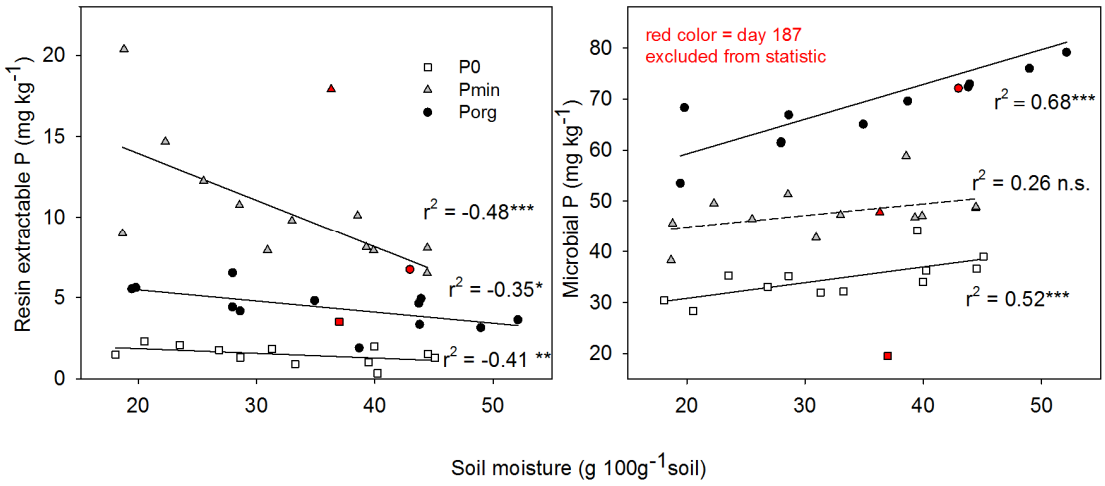

Figure 5.8: Relationship of resin-extractable and microbial P to soil moisture in the three P fertilizer treatments P0, Pmin and Porg in a mesic grassland managed at low intensity during the season 2009. Red symbols were excluded from statistical analysis. Significance levels are given as ns. = not significant, * = p<0.05, ** = p<0.01 and *** p<0.001.

5.5. Discussion

5.5.1. Yield and mean P_{res} and P_{mic}

The yield reached in Pmin and Porg was only 5 % below the expected yield (6.5 t ha^{-1}) reported for grasslands managed at low intensity (Flisch et al., 2009) . In contrast, the omission of P fertilization in P0 led to a severe yield reduction (Figure 5.6). Nevertheless, the lower yield and P uptake and higher soil total P found in Porg after 18 years of slurry application (Table 5.1) indicate that P availability was not similar although the estimated amount of P applied with slurry was similar to the mineral P fertilization.

The observed Porg was close to and Pmin tended to be higher than a P_{res} of 5.2 mg kg^{-1} observed under an extensively used grassland by Chen et al. (2000). But P_{res} was much lower in P0. Considering ranges of P_{res} from 1.0 to 58.8 found under grasslands of different management intensities and fertilizer input in Switzerland (chapter 3), the values observed here were in the lower third of the range. According to the classification presented in chapter three, mean P_{res} indicated a P deficient status for P0 and a sufficient status for Pmin and Porg. However, Pmin had values above the optimum of 6 mg P kg^{-1} whereas Porg lay below, indicating a risk for yield decline (e.g. when weather conditions are unfavorable during a season).

Although we used no correction factor (k_p), the P_{mic} stocks were within the range of 18 to 100 kg ha^{-1} reported by Brookes et al. (1984) for the upper ten cm of the soil with application of a k_p of 0.45. Mean P_{mic} concentrations in our study generally fell in the range of 20 to 88 mg kg^{-1} reported by Sarathchandra et al. (1984) for a range of grasslands, who used the k_p as Brookes et al. (1984). The higher P_{mic} concentration found in Porg compared better to ranges found by Sparling et al. (1994) under unfertilized (78 mg kg^{-1}) and fertilized (98 mg kg^{-1}) pastures. They also applied the k_p from Brookes et al. (1984).

5.5.2. Long term fertilizer effects on P_{res} and P_{mic}

Similar to other studies, we found significant positive effects of P fertilization on P_{res} and P_{mic} pools (Oberson et al., 1996; v. Lützow and Ottow, 1994). Organic fertilization was reported to result in lower P availability than mineral fertilizers when applied in the

same amount (Oberson and Frossard, 2005). In contrast, P_{mic} increases under organic fertilization (Oberson and Joner, 2005; Oberson et al., 1996; Oehl et al., 2001b). Carbon is known to be the main source of energy for the microbial biomass (Dalal, 1998). The lower P_{res} in Porg than in Pmin cannot only be attributed to the higher P_{mic} because other factors such as cation exchange capacity, pH and the relation of inorganic and organic P were also significantly different between Pmin and Porg (Table 5.2). The high variation of P_{res} and P_{mic} observed during the season and between the treatments reflects the big range of P_{res} and P_{mic} that can be found under grassland.

5.5.3. Fluctuations of resin extractable P

Differences of mean P_{res} between the treatments resulted from the long term P input and output of the treatments as reflected by mean total P in the soil (Table 5.2) and plant P uptake (Figure 5.6). Although absolute minima, maxima and amplitude of fluctuations in P_{res} differed between treatments, the synchronous fluctuations suggest that similar processes affected P_{res}.

Elevated P_{res} was always found during or after periods of relative dryness, indicating an effect of soil moisture. However, increased P_{res} values were not always accompanied by a reduction of P_{mic}, implying that the P release was not only from P_{mic}. In a drying and rewetting experiment under controlled conditions using soil from the same experimental site, Bünemann et al. (personal communication) observed significant increases of P_{res} only after drying to soil moisture below 150 g kg^{-1}. Since soil moisture in the field was never below 150 g kg^{-1}, we assume that drying and rewetting effects with P release from the microbial biomass were not the main driver of the increase in P_{res}. Other factors causing fluctuation in P_{res} could be plant P uptake, temperature, microbial grazing and physico-chemical processes (Denef et al., 2001).

Low soil moisture reduces the diffusional transport of P within the soil and thus potential P uptake by plants (Dunham and Nye, 1976). Jupp and Newman (1987) observed cessation of P uptake by *Lolium perenne* below a soil moisture of 200 g kg^{-1} soil (water potential of -0.6 to -0.8 MPa). Such low moistures were observed when the highest peaks in P_{res} were found at day 116, 156 and 246 (Figure 5.7). On the other hand, the three minima of P_{res} observed simultaneously in all treatments were found during periods when soil moisture was above 300 g kg^{-1}, suggesting unhindered P

uptake by the plants. Thus, we assume that reduced P uptake by plants was reflected in elevated P_{res} values while P_{mic} was not always reduced. The aboveground plant productivity of Pmin as shown in Figure 5.5 reflects a typical productivity curve for temperate grassland with two peaks and a growth reduction in summer (Gibson, 2009). The peak growth rates lay in periods when soil moisture allowed unhindered uptake by the plants. That a severe reduction in P_{res} was observed at both peaks of plant growth rate implies that plant P uptake plays a major role for the fluctuation of P_{res} during the season.

However, we observed a minimum of P_{res} at day 167 during the summer when the plant growth rate was reduced and soil moisture high. This suggests that another factor in addition to plant growth and P uptake affected the P_{res} pool.

5.5.4. Fluctuations in microbial P

Apart from occasional decreases of P_{mic} in P0 and Porg and an increase in all treatments in autumn, P_{mic} was relatively constant. The ranges of P_{mic}-fluctuation in P0 and Pmin were close to findings by Chen et al. (2003), who observed P_{mic} to fluctuate between 25 and 52 mg kg^{-1} under an extensively managed grassland. P_{mic} and P_{mic}-fluctuation was higher in Porg and compared better to findings of Perrott et al. (1990) in a highly productive pasture. Both studies found P_{mic} to peak in winter (July in the southern hemisphere) and to decline during summer. In our study no measurements were done in winter but we found high values in early spring and late autumn.

The main differences between treatments in seasonal fluctuation of P_{mic} were observed during the first half of 2009 (until approximately day 220). The reductions of P_{mic} were always observed during or shortly after periods when soil moisture was below 300 g kg^{-1}. Based on the laboratory drying-rewetting experiments mentioned above, a significant decline in P_{mic} occurs only after reduction of soil moisture below 150 g kg^{-1} (Bünemann, personal communication). Thus, P_{mic} reductions were caused by other processes such as the interaction with plants (Reynolds et al., 2003) or temperature (Grisi et al., 1998). The different P_{mic}-fluctuation in the treatments could be explained by effects of fertilization. In Porg we observed two severe P_{mic} reductions in the first half of the season which were accompanied by soil drying to below 200 g kg^{-1} and subsequent increases when soil moisture increased. At the same time no decreases were

found in P0 and Pmin. We assume that the competition of microbes and plants for available P was increased in Porg which had generally a higher level of P_{mic} than the other treatments. A partial dieback of microbial biomass could be a consequence of a plant induced shortage in P and result in the observed reduction of P_{mic}.

Another severe reduction in P_{mic} was observed in P0 at day 185 at the end of a dry period which was accompanied by a significant increase of P_{res} and the maximum mean daily T_{bg} of 24.4°C. The daily maximum at day 185 exceeded 30°C. Thus, we assume temperature and drought stress for the microbial biomass. A lower aboveground biomass could create less shading and cooling by transpiration and thus result in higher soil temperatures. This is supported by the fact that the decrease of P_{mic} and the increase in P_{res} were not found in the other two treatments. Since the T_{bg} was measured outside the plots for the whole site we cannot confirm this. Another explanation would be that the microbial biomass was more susceptible to drought stress because of P deficiency (Harris, 1981).

The increases of P_{mic} after the first and third harvest can probably be linked to belowground carbon input by plants. Fine root turnover is assumed to be a main source of organic material released into the soil by plants in grasslands (Olff et al., 1994) and root death was shown to be increased after cutting (Gibson, 2009). A boost of microbial biomass caused by release of carbon by root dieback or enhanced exudation after cutting of temperate semi-natural grassland was reported by Guitan and Bardgett (2000). We observed increased P_{mic} only when plant growth rate was low at day 163 and 288, reflecting a negative relationship (P_{mic}= -0.25*plant growth rate +13, r^2 = 0.37*, n = 12). The fact that P_{mic} was not significantly increased after the second harvest when plant growth rate was high further illustrates a possible competition between plants and microbes.

The similar P_{mic} fluctuation patterns in the three treatments in the second half of the season can be explained by the generally decreased plant growth rate after day 250 and the lowering temperatures and thus less competition for P_{res}. A distinct relationship between P_{res} and P_{mic} was not observed. However, the coefficients of variation in the treatments were always lower in the sum of P_{res} and P_{mic} than in the two P pools separately (data not shown), pointing to a counteraction. This counteraction might have been masked by the high variation in the field and the interactions of both P pools with plant growth and environment. This supports the statement by He et al. (1997) that a

combined use of both pools may improve information about plant availability of P. Nevertheless, the seasonal changes in P_{mic} showed that release and immobilization of P in the microbial biomass take place.

5.5.5. P fluxes and turnover

We calculated the P turnover by microorganisms as the total measured loss of P_{mic} divided by the average quantity of P_{mic} as proposed by McGill et al. (1986) for microbial biomass. If important decreases or fluctuations were missed or not measured to their full extent the turnover could be underestimated. Due to the structure of our sampling, we assume a reasonable cover of the main steps of seasonal fluctuation and thus reliable estimates of microbial P losses and turnover.

Chen et al. (2003) reported P_{mic} losses of 14 kg ha^{-1} yr^{-1}, whereas we found 3 to 5 times higher P_{mic} losses (Table 5.3) although we used no kp factor. These high P_{mic} losses resulted in turnover times of less than a year. In contrast, Chen et al. (2003) and Brookes et al. (1984) reported turnover times of more than a year. However, the variation in turnover time between soils is great (Brookes et al., 1984) and the estimation strongly depends on the measured P_{mic} releases. As discussed above, these releases may vary between seasons and changing climate conditions as well as with the management of the grassland. The low P release and thus lower turnover observed by Chen et al. (2003) may have resulted from the lower number of soil samplings and from the arrangement of soil samplings in a monthly pattern rather than based on weather conditions or management practices. The tendency for a faster turnover under P limiting conditions in P0 than in Pmin and Porg is in agreement with the observation by Oehl et al. (2001b) and further confirms the hypothesis by Magid et al.(1996) that P_{mic} cycling is faster in P deficient soils. Underlying mechanisms can be a higher susceptibility to environmental stresses such as drought and heat and thus a shorter lifespan (Harris, 1981) and an increased P uptake efficiency of microbes under P deficient conditions (Vershinina and Znamenskaya, 2002).

The higher P_{mic} in Porg than in Pmin is in agreement with many reports (Oberson et al., 1996; Oehl et al., 2001a). But the higher P_{mic} found under organic fertilization did not result in a higher turnover rate than in the mineral fertilized Pmin. The higher P_{mic} fluxes in Porg resulted from the higher P_{mic} pool which was turned over in the same

time. The P_{mic} fluxes in our study are in agreement with Brookes et al. (1984). They reported a range of P fluxes through the microbial biomass of 7 to 40 kg P $ha^{-1}yr^{-1}$. The P_{mic} flux in the organically fertilized Porg was similar to P fluxes of 35-36 kg P $ha^{-1}yr^{-1}$ found in pasture soils (Sparling et al., 1994).

Although the seasonal P_{mic} fluxes were always larger than seasonal plant P uptake we found significant effects of fertilization on plant P uptake. In P0 and Porg, however, plant P uptake was lower than in Pmin in spite of the larger P_{mic} pool and P_{mic} fluxes. This indicates that the higher P flux was not beneficial or not accessible to the plants and that a competitive situation between P_{mic} and plants for available P might have developed (for Porg only in spring). This underlines the importance to consider soil microbes and their activity when P availability is estimated.

5.6. Conclusion

The P fertilizer regime had a strong impact on P_{res}, P_{mic}, their interaction and their fluctuation during the season. A higher P_{mic} turnover rate under P deficiency was observed but not significant, which could be explained by the high variation of P_{mic} in the field. The higher P_{mic} pools in the mineral or organically fertilized treatments resulted in higher P fluxes. Regardless of the treatment, turnover and fluxes of P_{mic} were higher than plant P uptake but not accessed by plants. A competition for available P between plants and microbial biomass seems to be indicated. The potential of P_{mic} to withhold available P during times of high P demand of plants or dry conditions was shown and may thus affect the plant yield as observed in Porg.

Resin-extractable P was mainly determined by plant P uptake and thus indirectly affected by soil moisture and temperature. Factors affecting microbial P were more difficult to determine, but an interaction of plant growth (carbon input and competition for P_{res}), soil moisture and temperature is probable.

Practical consequences could be that organic fertilization should be exchanged by mineral fertilization (for a while) to reduce the competition effect (no carbon) and increase P availability. For the measurement of P fluxes and turnover it seems appropriate to follow the important steps of management and weather changes during the season. In that way major changes of P_{mic} as a result of the changing environment

for the microbes in the soil can be observed. We underline the importance of the microbial P pool for P cycling.

6. General discussion

6.1. Overview

In section 6.2. of this general discussion I will return to the dataset presented in chapter two. In addition to the plant P indicators assessed in relation to N and K, many other nutrients were analysed in this study but could not be presented and discussed in chapter two. Here I will present data for Ca, Mg, S (only first harvest 2009), Fe, Zn and Cu (Table A.2) and discuss them briefly with respect to critical values from the literature and calculated DRIS indices (Table A.3).

The next section (6.3.) compares the five soil P indicators from chapter three and five, evaluating if soil P accumulation can be avoided by a valid assessment of surplus P nutrition status in permanent grasslands. Further, I will integrate the thresholds for determination of soil P status established in chapter three and five in a five-step model as used in the Swiss fertilizer recommendation (Flisch et al., 2009).

In section 6.4. of the general discussion I will address the effects of weather conditions on the P availability and practical consequences. In chapter five we found that the interaction of P_{res} and P_{mic} potentially affects P availability and thus plant growth and P nutrition status. This will be further investigated with data from chapter three and four which was not yet presented.

In section 6.5. a synthesis for plant and soil indicators will be presented and a scheme for the combined synergistic use proposed. It is indicated where additional information such as weather condition could support the interpretation of plant and soil P indicators.

At the end of the general discussion I will present some questions for future research on nutrient management in grasslands and the conclusions of my thesis.

6.2. Plant mineral nutrient status

6.2.1. Relationships to other nutrients

Many tools for diagnosing P nutrition status (and that of other nutrients) with plant tissue analysis have been developed (reviewed in Black, 1993). In chapter two, we derived the interpretation schemes for P concentration and confirmed the applicability

of the PNI and its interpretation in grasses. Thus, they are ready to be used as a standalone indicator for sward P diagnosis or to calibrate and assist soil P analysis in grasslands. For the nutrient ratios and DRIS we identified the need to refine the critical and norm ratios used for interpretation and calculation, respectively. Further, we showed that P indicators that integrate other nutrients were more strongly related to yield than using just P concentration. However, to identify nutrient co-limitation, a set of nutrient indicators or "ideally" the DRIS indices were necessary. To address this, we also analysed other nutrients such as Ca, Mg, S (first harvest 2008), Fe, Zn and Cu (Table A.2). These nutrients can be included to identify the nutrition status for other elements and to reveal nutrient co-limitations of the investigated grasslands.

6.2.2 Additional information obtained by DRIS and nutrient concentrations

Our data set was not fully appropriate to derive the norms for all the nutrient ratios used for the DRIS calculation. Thus, we used the published norms for perennial ryegrass, cultivar Talbot (Bailey et al., 1997b) to calculate DRIS indices (Table A.3). A short description of the results can be found in the annex after Table A.3.

For N we found no indication of severe deficiency. Although N concentrations were low in the first harvest of some N1 fertilized treatments, this was not reflected in sward yield and DRIS N. This can most likely be explained by a dilution effect due to the high growth rate in spring (Bélanger and Gastal, 2000). However, we identified it as a potential co-limitation with P and K in W-N0P0K0 and as K-deficiency induced N limitation (Minotti et al., 1968) in W-N1P1K0.

The stronger K limitation of plant growth in the treatment V-N1P0.5K0.5 hypothesized in chapter two was confirmed by the higher DRIS P than DRIS K in all treatments in V (Table A.3). The positive DRIS P index for VN1P0.5K0.5 indicates that P was not limiting and thus the observed yield reduction was mainly caused by the K limitation. Consequently, K limitation should be relieved by fertilization. For Ca and Mg we found no evidence for a yield limiting effect in our study although some indicators were low. The observations for Fe led to similar conclusions. For these minerals the indicators do not seem well defined for the diagnosis of nutrient status in grasslands and could be a target of further research.

Although low, the observed concentrations for Zn, Cu and B were still within the reported sufficient ranges from Whitehead (2000). For Mn concentrations, we found low values in the intensively used grasslands in B, with an exceptionally low value in N2P2K1org II. This could be caused by environmental conditions (bad drainage and a shadowed exposition).

For S we found a potential co-limitation with other nutrients in the two treatments in W-N0P0K0 and W-N1P1K2org but could not confirm it because we only analysed S in the first cut.

6.2.3. DRIS norms and nutrient ratios need a broader data range for evaluation

Although the DRIS norms reported for perennial ryegrass (Bailey et al., 1997b) proved applicable in the grass fraction of mixed species grasslands the absolute values might not have been always precise. We observed a difference between the mean ratios in our study and the norms derived for ryegrass in a pot experiment. An example are the lower mean ratios for N:P and K:P in our experiment (7.5 and 7.3) and the norm ratios (9.0 and 8.8) in chapter 2 (Figure 2.3). The difference indicates that the DRIS norms (Figure 2.4) potentially can, and should, be refined for agricultural grasslands under field conditions. This is in agreement with Bailey et al. (1997a) who also observed a need to adjust DRIS indices when applied to field samples. Therefore, a greater database including more differentiated nutrient inputs or field observations is needed.

In Figure A.2 the data for N:P ratios in grasses from our study were combined with N:P ratios from grasses of a similar growth stage from the “DOK” trial in 2007 (Oberson, personal communication). Using this dataset identifies a norm ratio at seven with a standard deviation of 2.2, confirming our suggestion (chapter 2) that the norms proposed by Bailey et al. (1997a) might be too high. In addition, the interpretation range for sufficient N and P nutrition is refined to 5 to 9 in grasses. This indicates that a yield limitation due to deficient N or P is probable for N:P ratios below 5 or above 9, respectively, for agricultural grassland.

With this example I want to show that by combining datasets, which include nutrient concentrations of the grass fraction (vegetative growth stage) and sward yields, the DRIS norms and nutrient ratio interpretation can be refined with relatively little effort

and cost. Subsequently this powerful tool for holistic nutrient diagnosis would be applicable for agricultural permanent grasslands.

6.3. Estimating P availability with soil analysis

6.3.1. Comparison of the methods

In chapters three and five we investigated the five soil P indicators P_{ox}, E_{1min}, P_{res}, P_{CO2} and P_{AAE} as related to plant yield and P nutrition status. The coefficients of correlation can be found in the annex (Table A.5). The relationships between the five soil P indicators and the plant indicators were well described by exponential functions with a maximum. We established thresholds for soil P status classes defining deficient, sufficient and surplus P availability. For all five soil P indicators it became apparent that at the lower range the soil P indicators were less reliable than the plant P indicators (e.g. PNI in grasses) in identifying sward P nutrition status. Reasons were 1. a low resolution of the soil indicators in the low part of the response curve and 2. the high range of relative yield and P nutrition status indicators related to low soil P values. These constraints were relatively small for P_{res} and P_{AAE}. For P_{CO2} the relationship was improved for the investigated soils when clay content or soil buffer capacities were considered. It can be assumed that the use of co-factors potentially improves the determination of P nutrition status for P_{ox} and E_{1min}, but that was not aim of the study. It also needs to be considered that measuring additional factors always increases the costs for the soil test. Seeing that P_{res} and P_{AAE} showed the strongest relationships to relative yield, they should be the methods of choice if only one measurement is taken. However, determination of P_{res} on field moist soils disqualifies it as a routine test. The use of P_{res} on dried soil is widely practiced but thresholds for the soil P status classes need to be evaluated. E_{1min} is not applicable as a routine soil test for health and environmental reasons, but it is often used to study P cycling and other processes affecting P availability (Frossard et al., 2000). The here established thresholds for E_{1min} (Table 3.3) may be used when permanent grasslands are studied.

6.3.2. Only soil analysis identifies phosphorus accumulation

Fertilization of P on P accumulated soils is inefficient because no yield response is expected and P losses from the field may increase. Accumulation of P in the soil surface layers cannot be linked to losses by leaching or drainage per se (Sinaj et al., 2002). But it increases P losses by surface runoff (McDowell and Sharpley, 2001) and may lead to P accumulation and decreased P buffer capacity in the subsoil (Chrysostome et al., 2007; Ulén and Jakobsson, 2005). Although we did not investigate P losses directly, we found that P accumulation was still apparent in the subsoil (20 -40 cm) of the surplus fertilized treatments. In the two surplus-fertilized treatments in B (N2P1K1org I+II), the soil P indicators in the subsoil layer showed values which were still sufficient for plant growth (Table 3.2) according to our proposed thresholds. In a companion project it was shown that P in the surface run off from N2P2K1org was higher than in the runoff from N1P1K2org after similar simulated slurry applications and rain events (Hahn, personal communication). Thus, we observed a highly inefficient fertilizer application and the indication of increased risk for P losses in N2P2K1org I+II. To reduce P accumulation and P losses a cessation, or strong reduction, of P fertilization has been proposed (Frossard et al., 2005; Schärer et al., 2007; van der Zee et al., 1992).

In chapters three and four we stated that an indicated surplus plant P can occur even when soil P availability is only sufficient (e.g. N1P1K0 and N1P1K1). Such observations may be explained by the fact that not all plant available P sources and soil depths are accessed by soil analysis. Alternatively, the weather and growth conditions could have been favourable for P uptake and P mineralization in the soil. However, cessation of P fertilization when soil P is sufficient could lead to unwanted reduction of plant P nutrition status. In contrast, when P is truly accumulated in the surface soil P fertilization should be reduced, or even ceased. Such a drastic measure may be necessary because significant reductions of soil P cannot be achieved in the short term (Schärer et al., 2007). In six out of seven long term fertilizer experiments, no yield response was observed even after nine years of withholding P fertilization (Gallet et al., 2003a). Thus, we recommend that a P surplus identification by plant analysis should be supported by soil P indicators. In this way a recommendation of P fertilizer cessation can be justified as necessary and a wrongly reduced P fertilization avoided.

6.3.3 Interpretation schemes for soil P indicators

In principle, a classification of soil P availability into deficient, sufficient and surplus categories should be adequate for fertilizer recommendation. However, today in many European countries a five step classification is used (Flisch et al., 2009; Kerschberger et al., 1997; MAFF, 2000). This classification is widely known and accepted by farmers and used by extension services for fertilizer recommendation. Thus, for a refined interpretation, the thresholds established in our study should be adapted to such a classification (Table 6.1). For such a classification we additionally use the thresholds middle and maximum which reflect an optimum growth and P nutrition status and the absolute maximum of the response curves, respectively (see chapter 3 and 5).

Table 6.3: The three plant P nutrition status classes sufficient, deficient and surplus as adapted to a five class model for soil P classification for fertilizer recommendation. Borders between classes are defined by thresholds derived in this study.

Soil P class	**A**	**B**	**C**	**D**	**E**
Plant P nutrition	deficient	sufficient	sufficient	surplus	surplus
Threshold to higher soil P class	low	middle	high	maximum	---
P fertilization recommendation	Strongly increased	Slightly higher than plant P uptake	According to plant P uptake	Reduction	Stopped

In Switzerland a five step interpretation is used for classification of P_{CO2} and P_{AAE} . However, recent studies have emphasized that the underlying thresholds result in lower soil P classes than suggested by the actual plant P nutrition status (Frossard et al., 2004; Neyroud and Lischer, 2003). This is in line with our observations in agricultural permanent grasslands (chapter 4). The thresholds for P_{CO2} and P_{AAE} observed in our study may be helpful to refine the soil P interpretation schemes and thus improve P fertilizer use. Because time and investigated field situations were limited in our study, these observations need to be confirmed for a broader range of conditions. This should include other soils, management intensities and environmental conditions.

6.4. Fluctuations of available phosphorus

6.4.1. Interaction of many factors

In chapter five we observed strong variation of P availability during the season and differences of that variation between mineral, organic and unfertilized treatments. We identified the major factors controlling the available P pool to be plant growth, the microbial P pool (P_{mic}) in the soil and environmental conditions (mostly soil moisture). Although this is not new, our observations may add to the understanding of P availability and cycling in grasslands under field situations. In addition, our observations may have practical consequences. Sampling time is very important when analysis of field moist soil is planned or P fluxes and turnover are investigated. For fertilizer recommendation an early soil sampling before fertilizer application is generally suitable. But if the diagnosis of constraints for plant P uptake (competition with microbes for P or dry weather) during the period of highest plant P demand is aimed, late spring is a good sampling period. However, samples should not be taken after harvests or long dry spells. For example, we observed increased P_{res} after dry periods and reduced P_{res} after harvests. Sampling at such moments could create an over- or underestimation of the average P availability during the season. When P_{mic} fluxes and turnover are investigated the sampling should be oriented along major changes of the environment for the microbes. Such changes are caused in grasslands by management (e.g. fertilizer application, sward cutting) and weather conditions (e.g. dry periods or heavy rains). Such constraints are less important for analysis using dry soil because the sources for variation (e.g. P_{mic}) are drastically reduced or removed (see discussion chapter 5).

Seeing the strong influence of soil moisture on P availability, climate data can support the interpretation of P availability in the field and subsequent management considerations. The observed P releases after periods of relative drought might not be plant available because of too low soil moisture for P transport in the soil and plant P uptake (Dunham and Nye, 1976; Jupp and Newman, 1987). Such a shortage can only be relieved by irrigation. But irrigation of grasslands might not be an economic or practically applicable measure. Where irrigation is not feasible or cannot be realized, yield reductions must be accepted. With increasing incidences of summer drought as predicted in climate change scenarios (Schär et al., 2004) it might become necessary to

adjust fertilizer recommendation and management practices in regions with a high risk. Models (or maps) using climate data in combination with the water holding capacity of soils have been used to evaluate potential plant productivity for larger areas (Piedallu et al., 2011). Similar approaches including spatial P availability may result in useful tools for precision farming and site specific fertilizer recommendation (Betteridge et al., 2008) or when potential land use is evaluated.

6.4.2. Microbial phosphorus – an underestimated factor?

In addition to environmental factors and plant growth, we found microbial P in the soil to be a major factor affecting P availability. Microbes in the soil have been found to buffer or replenish and immobilize available P in the soil solution (Oberson and Joner, 2005). They are important mediators for P cycling (Oberson and Frossard, 2005) and may be beneficial for plant P availability and uptake in the case of mycorrhiza (Smith and Read, 1997). In an incubation study we labelled soils taken from the three P fertilizer treatments described in chapter five with ^{33}P (Keller, 2010). We observed enhanced ^{33}P recovery in P_{mic} in the P deficient soil (~40%) when compared to soils with sufficient P nutrition status (30%) which were fertilized with mineral or organic P fertilizer, respectively. The turnover rate of P_{mic} in the field (Table 5.3) was also higher in the P deficient soil than in the two P fertilized soils during the season in 2009. Although the P_{mic} stock in the 0-5 cm soil layer was always higher than the annual sward P uptake (Table 5.3), there was no indication of a beneficial effect on plant growth as often suggested in the literature (reviewed in Oberson and Joner, 2005). Further, we found indications that a high microbial P pool in the soil may act as a competitor for available P when plant growth rate is low and conditions for P transport and plant P uptake in the soil are suboptimal. He et al. (1997) suggested that the integration of P_{mic} for P availability studies might improve the estimation and reliability of P availability for plants in soils. In chapter five we found no significant relationship between plant P nutrition status and P_{mic} or the combination of P_{mic} and P_{res}. However, in that study we had only three treatments and only considered P concentrations in grasses. Using the data from the plant and soil P indicators survey (chapter 2 and 3), where we also measured P_{mic}, showed no distinct relationship between P_{mic} and plant P nutrition status when P_{mic} was used alone. For the sum of P_{res} and P_{mic} we observed

specific relationships for each location (data not shown). But the ratio P_{res}:P_{mic} (Figure 6.1) revealed a strong exponential relationship to plant P nutrition status as described by PNI over all treatments and locations. When P_{res} was lower than 10% of P_{mic} the plant P nutrition status was significantly reduced. This suggests that microbes may act as a competitor for P under low soil P conditions. This observation underlines the importance of P_{mic} for the interpretation of plant P availability in permanent grasslands.

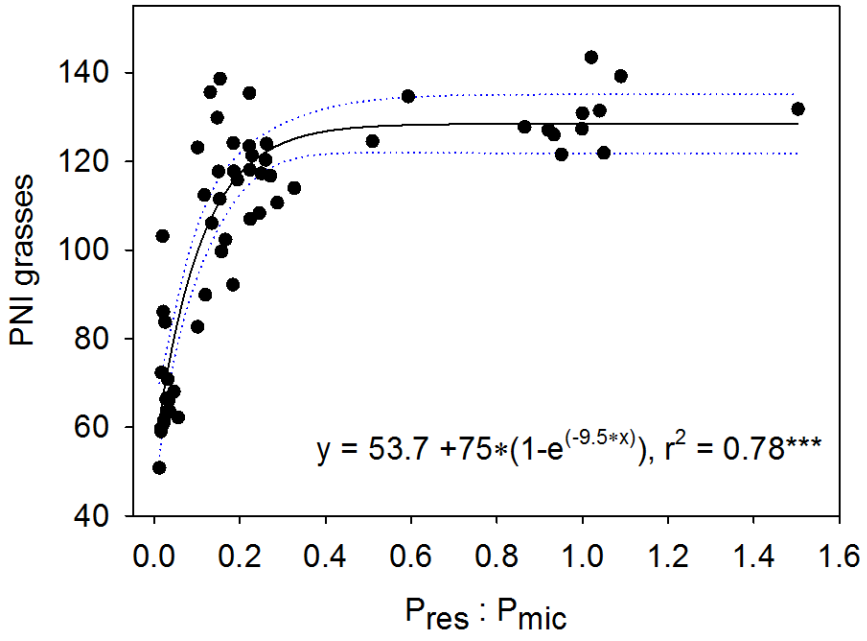

Figure 6.1: The P nutrition index in grasses (PNI) as related to the ratio of P_{res} to microbial P (P_{res}:P_{mic}). Trend lines with ± 95% confidence range are shown (n = 57).

6.5. Plant, soil and environmental indicators in permanent grasslands

6.5.1. A synthesis improves the phosphorus status assessment

In the previous chapters we defined thresholds for plant and soil P indicators (Tables 3.3 and 4.2). Each method can be used separately for P nutrition status diagnosis and for fertilizer recommendation in permanent grasslands. However, neither plant nor soil analysis is perfect to assess the P nutrition status. Sometimes they result in contradictory results. Such observations reflect the known advantages and disadvantages of both methods (Marschner, 1995). A combination of both soil and plant P indicators improve the assessment of the P nutrition status. However, to understand the underlying processes and reasons behind the observed P nutrition status

it is necessary to integrate other factors such as co-limitation by other nutrients (Figure 2.3), environmental conditions and microbial P. In figure 6.2 I suggest a procedure how to use the advantages of each method in an integrated diagnosis scheme.

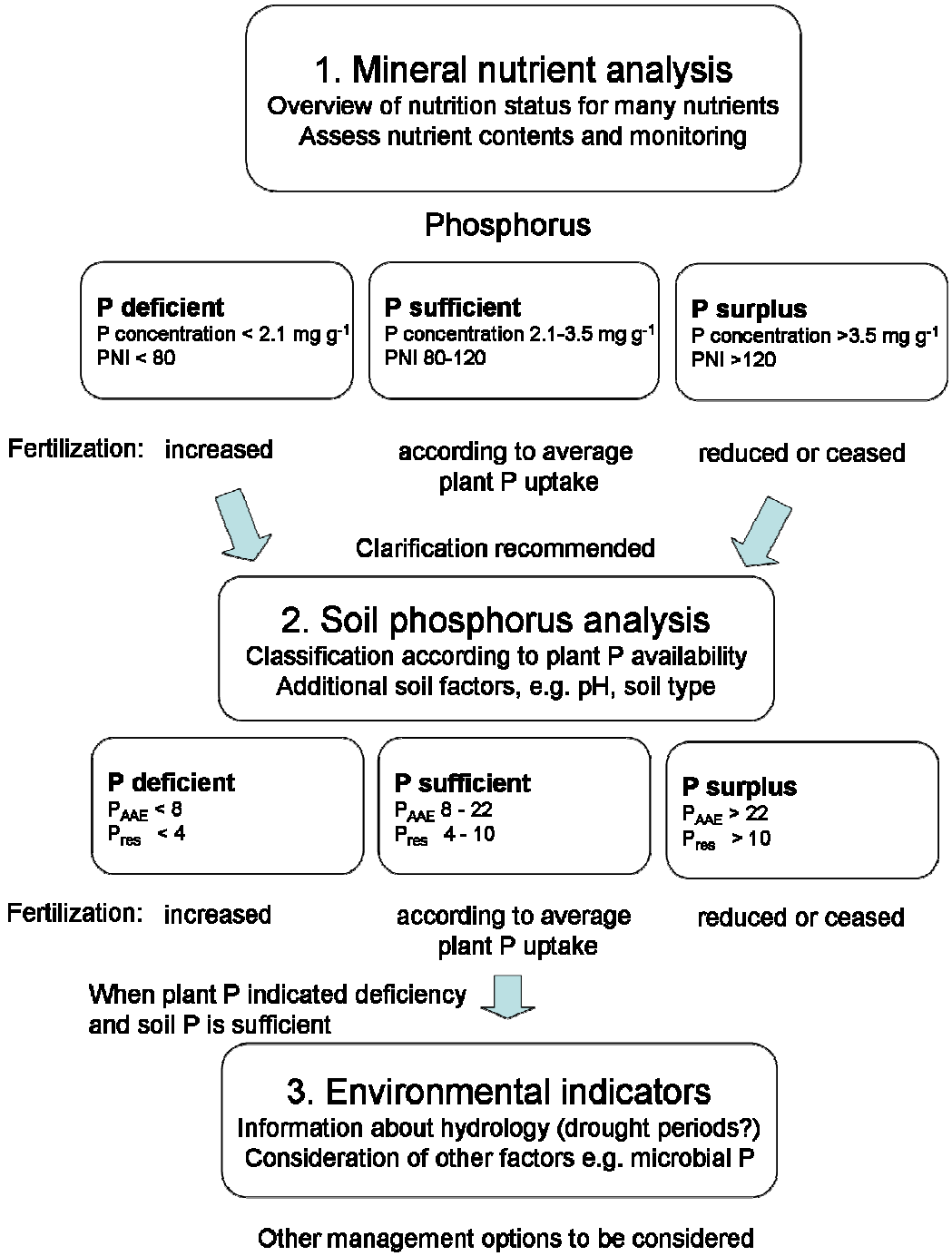

Figure 6.2: Integrated diagnosis scheme for sward P nutrition.

The following steps for P nutrition status diagnosis imply that the investigator does not know anything about the grassland under investigation.

1. As the first step for nutrient diagnosis of grasslands a plant nutrient analysis should be conducted for as many nutrients as possible. An intensive nutrient survey is relatively cheap and easy for plant analysis using total element analysis (ICP and CNS). The samples should be taken in an early growth stage of the grassland and only grasses should be collected. A diagonal walk over the sward, sampling a hand full of grasses every 10 steps should be sufficient and representative (simple and fast). The analysis result should be interpreted for each nutrient using the best available indicator and in relation to the other nutrients to reveal potential co-limitation. Assuming that a sufficient P nutrition status was identified (PNI 80-120) the fertilizer recommendation would be according to average P uptake by the investigated sward. For deficient or surplus indicated swards an increased or reduced P fertilization would be recommended.

2. This decision should be justified by an additional soil analysis to explore the reasons for P deficiency or identify the severity of P accumulation. The soil analysis should include basic parameters affecting P availability, such as soil type, structure and pH, and a routine soil P test with reliable interpretation scheme. When a deficient soil P status is indicated the increased P fertilization is justified. When a sufficient P nutrition status is found, the P availability must be constrained by other factors such as pH, soil structure, co-limitation with other nutrients (step 1) or bad environmental conditions for P uptake (step 3). In that case, the limiting factor should be eliminated before considering P fertilization. When a surplus P nutrition status is found fertilization should be reduced or ceased. When soil P is still below the maximum of the response curve (class D, Table 6.1, see also Table 3.3.) a reduced fertilization might be appropriate. But when exceeding the maximum (class E) the P fertilization should be ceased to drastically reduce the risk of P losses and minimize inefficient fertilizer use.

3. In case of deficient plant P (step 1) and sufficient soil P status (step 2), additional clarification is needed. A reduced P availability due to bad environmental conditions can be checked with meteorological data for the period before plant sampling. Such data are available for many regions in Switzerland by means of MeteoSwiss (IDAWEB, 2009). When the period before sampling was very dry fertilization should be according to P uptake, assuming that this was a unique event. When the drought period is repeated

every year the management should be reconsidered. When irrigation is not an option (most likely for grassland) the fertilizer recommendation should be reduced to the actual P uptake. Otherwise P fertilization is inefficient and there is a risk for P accumulation.

Of course not all the steps need to be always applied. Each step and tool can be used independently but the reliability might be lower. When soil analysis is directed by law as in the Swiss direct payment scheme (BLW, 1998), plant analysis can be used to assist nutrient diagnosis and for quality control of the produced forage. Thus, information about nutrients which are not covered by soil analysis (S, micronutrients) can be derived and imbalances in the plant nutrition avoided.

6.5.2. Open questions and need for further research:

1. The base for calculation and interpretation of DRIS and for many nutrient relationships should be refined to allow a general use for nutrient diagnosis in permanent grasslands.

2. Standard methods for plant analysis and their prices should be evaluated according to practical applicability. Such information may give evidence for the applicability and help to convince practitioners.

3. Soil analysis is well established but interpretation schemes should be refined with respect to fertilizer use efficiency and risk for P losses

4. Weather-induced P deficiency in permanent grasslands in Switzerland is not yet a topic for nutrient management but might become important in the future with increasing incidences of drought events. Potential effects on yield and management options such as irrigation or the use of drought tolerant species and cultivars should be evaluated

5. The role of microbes for P availability is not yet well understood. Especially the microbial release and immobilization of P and its relevance for plant growth and the transition between symbiosis, mutualism and competition with changing environmental conditions should be addressed by further research.

6.6. Conclusion

- For the use of plant mineral analysis, we recommend analysis of the grass fraction. The phosphorus nutrition index was found to be a very good and reliable indicator for plant P nutrition status of grasslands. Although less reliable for the identification of surplus P nutrition, the P concentrations in grasses are applicable as well.

- The multi-element approaches using nutrient ratios or DRIS proved to be useful tools when a holistic investigation of plant nutrition status is needed. However, we found that the basis for calculation and interpretation is not yet satisfyingly established for permanent grasslands. However, such a refinement can be achieved by integrating additional data and would improve nutrient status diagnosis with plant analysis.

- Both plant and soil P indicators are valuable tools to determine the P nutrition status of permanent grasslands and can be used for fertilizer recommendation. With the thresholds established in this study, the investigated indicators can be interpreted for permanent grasslands under field conditions. A combination of plant and soil indicators increases the reliability of the information (and the resulting decisions).

- For the soil P indicators resin extractable P seemed the best to identify P availability in the soil. In connection with this analysis, microbial P can be estimated and a combination of both may improve the interpretation for plant availability.

- P_{AAE} and P_{CO2} were shown to be applicable soil P indicators but additional parameters affecting P availability in the soil need to be considered for a reliable interpretation, particularly for P_{CO2}. The comparison of the established thresholds with the actual interpretation scheme suggests that the actual ranges for interpretation in the Swiss fertilizer recommendation can potentially be reduced.

- When processes such as P turnover and P cycling are studied under field conditions, plant, soil and environmental indicators should be integrated.

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Annex

Description of botanical composition

Les Verrières is a subalpine grassland with fresh to moist hydrology and slightly acid soil reaction. According to Klötzli et al. (2010) the vegetation can be described as a *Festuco-agrostietum* association (species-rich grassland with 30-50 flowering species). The main species we found were: *Agrostis capitalaris, Anthoxantum odoratum, Festuca rubra agg., Luzula campestris agg., Achillea millefolium, Plantago lanceolata, Hypericum ssp.* and *Leucanthemum vulgare agg.*. A transition to a *Arrhenatherion, Polygono-Trisetion* association (increased nutrient input and cutting) is indicated by: *Dactylis glomerata, Trisetum flavescens agg., Poa trivialis, Lathyrus pratensis, Lotus corniculatus, Trifolium pratense, Vicia cracca, Alchemilla vulgaris, Centaurea jacea, Polygonum bistorta, Ranunculus acris, Rumex acetosa, Taraxacum officinale* and *Veronica ssp*. (only species present in all investigated treatments). The generally low agronomic value of this sward is due to the short vegetation period resulting in low dry mass production (2 cuts per year) and the late first cut which produces forage of low quality (low protein and energy but high fiber content).

Watt is a naturally well drained mesic grassland habitat (dry periods possible in summer) and slightly acid soil reaction. The vegetation is an Arrhenaterion elatioris association (30-40 species) reflecting the influence of fertilizer input. Characteristic species (found in all treatments): *Arrhenaterum elatius, Anthoxanthum odoratum, Cynosurus cristatus, Festuca pratensis*/*rubra agg., Holcus lanatus, Trisetum flavescens, Lotus corniculatus, Trifolium pratense, Vicia cracca, Campanula patula, Crepis biennis, Galium album, Plantago lanceolata, Ranunculus acris, Rumex acetosa*. Species frequently found (most plots/treatments): *Centaurea jacea, Knautia arvensis, Leontodon hispidus, Leucanthemum vulgare, Salvia pratensis* and *Tragopogon pratensis*. Species indicating an increased nutritional status: *Dactylis glomerata*; *Lolium perenne, Trifolium repens, Vicia sepium* and *Taraxacum officinale* (present in all treatments). In 2006 a grassland flower mixture was sown in (for experimental reasons) but did not show significant germination in the year of investigation 2007. The agronomic value of the sward can be substantial with relatively high dry matter yields and forage quality. Only the late first cut is of minor forage quality.

The location in Baldegg is characterized by a warm climate and good hydrology with high nutrient inputs and 4 to 6 cuts per year (with early/long cultivation background). Mean species number in the investigated grasslands is 18-25 and they were used only for silage. Vegetation can be characterized as high yielding *Trifolio-Lolion* association. Characteristic species are *Lolium perenne* (always the main grass), *Lolium multiflorum, Dactylis glomerata, Poa pratensis/annua, Trifolium repens, Rumex obtusifolius,* and *Taraxacum officinale* (present on all swards). Also observed were *Alopecurus pratensis, Festuca pratensis/rubra agg., Cardamine pratensis agg., Capsella bursa-pastoris, Cerastium caespitosum, Ranunculus ssp.* and *Veronica ssp.*. These grasslands are of high agronomic value because of their high biomass production and generally high forage quality produced by early cutting (silage).

Additional information on plant analysis

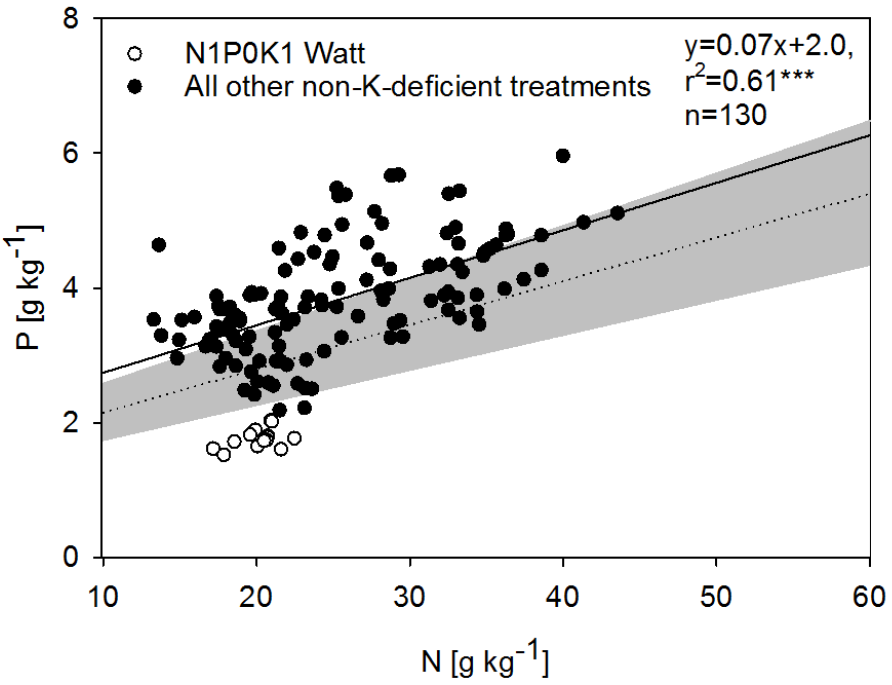

Figure A. 1: Relationship of N and P concentrations in all non-K-deficient treatments (n=130) in the above ground biomass of the grass fraction sampled at growth stage 3-4 (spike appearance to pushing) of *Dactylis glomerata*. The solid black line represents the linear regression line for all not K deficient samples excluding N1P0K1. The dotted line represents the critical curve (P=0.065N+1.5, N and P in g kg^{-1}) published by Duru et al. (1997). The grey field marks the range of PNI between 80 and 120 proposed to be satisfactorily P supplied. The coefficient of correlation was calculated using the Spearman correlation (*** = $p<0.001$).

Table A.1: Ranges of sufficient nutrient concentrations in permanent grasslands and selected grassland species from five literature sources.

Source	plant	N	P	K	S	Ca	Mg	Fe	Zn	Cu	Mn	B	Na
		mg g^{-1}						mg kg^{-1}					
Bergmann (1993): sufficient mineral nutrient concentrations, ca. 5cm aboveground beginning of flowering													
	Average grasses	26-40	3.5-6	20-30		6-12	2-6		20-70	5-12	35-100	6-12	
	Dactylis glomerata	28-4	3-5	25-35		6-12	1.5-3		20-50	6-15	40-100	6-12	
	Lolium perenne	30-42	3.5-5	25-35		6-12	2-5		20-50	6-12	40-100	6-12	
	Festuca pratensis	26-38	3-5	21-35		6-12	1.5-5		20-50	6-12	35-100	6-12	
	Trifolium pratense	25-40	3-6	18-30		10-20	2.5-6		25-70	7-15	35-100	25-60	
	Trifolium repens	28-40	3-5	17-25		10-20	2.5-6		30-80	6-15	35-100	25-60	
Breuer et al. (2003): sufficient mineral nutrient concentrations, beginning of flowering													
	Average forage grasses	24-40	2.5-6.0	20-40			1.0-6.0			5-15	28-140		
Whitehead (2000): typical concentration ranges at leafy stage of growth													
	Lolium perenne	20-35	2-6	15-35	2-5	4-8	1-3	100-200	15-60	3-15	30-300	2-8	500-4000
	Trifolium pratense	34	2.7	20.7	2.1	18.4	2.1	85	24	7.4	44	27	400
	Trifolium repens	35-52	2-6	15-35	2-5	10-20	1.5-4	100-300	20-40	5-12	30-200	10-40	500-4000
Barker and Pilbeam (2007):optimum or sufficient range													
	Forage grasses	20-32		25-35	2-3.2							10-50	
	Legumes	38-45		17-30	2-3.2								
	Lolium perenne		2.8-4.4	25-35			1.6-3.2			4.5			
	Dactylis glomerata		2.3-2.8	25-35			1.5-3						
	Trifolium repens		2.5-3	17-25			2-6			12			
	Trifolium pratense		2-8	18-30			2-6	93		16.1		20-60	
Flisch et al. (2009): Ranges of nutrient element concentrations in bulk samples													
	Intensive grassland	22-27	3-4	20-33		8-12	2-3						
	Low intensity grassland	13-22	2.8-3.5	12.5-25		8-12	1.6-2.6						
	Grasses pure stand					4-6	1.5-3.1						
	Legumes pure stand												

Table A.2: Mean mineral nutrient concentrations in the grasses, legumes and forbs in the first regrowth 2008 sampled at vegetative growth stage.

Location	Treatment	Mineral nutrients											
		N	P	K	S	Ca	Mg	Fe	Zn	Cu	Mn	B	Na
Grasses		mg g^{-1}						mg kg^{-1}					
Les Verrières	N1P0K0	18.4	1.8	13.4	3.0	3.8	1.7	52	35	4	303	4	470
	N1P0.5K0.5	18.1	2.5	17.3	2.7	3.3	1.5	56	27	4	352	3	141
	N1P1K1	18.8	3.3	21.9	2.6	3.4	1.4	59	33	5	388	4	95
	N1P2K2	17.9	3.3	22.9	2.6	2.8	1.3	70	30	5	412	3	135
Watt	N0P0K0	14.9	1.6	13.0	1.7	3.9	1.5	33	36	3	262	6	214
	N1P0K1	18.9	1.7	23.5	2.0	2.4	1.3	27	26	7	317	5	140
	N1P1K0	16.8	3.1	15.1	2.9	3.7	2.0	25	26	3	228	6	343
	N1P1K1	19.0	3.8	26.7	2.5	3.2	1.7	26	28	4	301	6	196
	N0P1K1	17.0	3.6	24.0	2.3	3.0	1.5	24	27	4	305	4	279
	N1P1K2org	21.1	2.6	26.0	1.8	3.6	1.5	33	30	5	101	6	274
Baldegg	N1P1K2org	18.2	3.9	27.6	3.9	4.2	1.4	24	18	5	35	5	181
	N1P1K2org/min	24.8	4.9	33.2	2.3	3.5	1.7	35	27	7	58	4	1016
	N2P2K1org I	26.0	5.3	34.5	3.9	5.0	1.7	34	41	7	56	8	1055
	N2P2K1org II	25.7	4.8	30.8	2.1	5.1	1.6	31	31	6	21	5	543
Legumes													
Les Verrières	N1P0K0	38.2	3.0	8.3	2.3	11.4	3.2	80	47	10	68	26	58
	N1P0.5K0.5	35.2	3.0	10.7	1.9	13.9	3.3	109	38	9	87	25	59
	N1P1K1	36.6	3.6	14.7	1.9	11.7	2.9	87	35	8	66	21	73
	N1P2K2	36.0	3.8	25.2	1.9	12.1	2.9	96	40	8	99	17	57
Watt	N0P0K0	33.9	3.2	12.2	2.8	13.3	2.7	68	44	11	91	27	226
	N1P0K1	32.4	2.0	18.6	2.5	10.0	2.7	60	41	13	94	22	250
	N1P1K0	40.5	4.5	11.8	3.1	11.2	3.3	85	49	10	88	32	733
	N1P1K1	36.3	3.5	20.7	2.2	13.6	3.6	70	33	8	86	21	361
	N0P1K1	32.0	3.4	20.3	2.1	14.0	3.7	69	28	8	104	21	274
	N1P1K2org	35.9	3.1	22.1	1.9	13.9	3.4	73	42	11	48	25	357
Baldegg	N1P1K2org	44.6	5.1	25.5	3.0	16.7	2.3	86	21	8	31	29	802
	N1P1K2org/min	39.1	5.0	24.8	2.2	13.0	2.5	119	31	10	37	26	3843
	N2P2K1org I	36.7	3.9	34.3	9.7	16.9	2.4	93	31	9	47	31	446
	N2P2K1org II	30.5	3.8	34.5	8.6	16.8	2.3	81	31	8	23	28	674
Forbs													
Les Verrières	N1P0K0	17.6	2.0	10.5	2.9	9.8	3.4	109	38	7	96	22	101
	N1P0.5K0.5	17.4	2.7	15.4	3.4	9.6	3.1	97	37	7	106	20	138
	N1P1K1	17.8	3.3	17.7	3.2	7.9	2.8	96	40	8	115	18	135
	N1P2K2	17.2	3.3	23.9	3.1	6.9	2.2	117	35	6	145	18	114
Watt	N0P0K0	17.7	2.4	12.1	2.6	12.9	3.0	69	58	7	109	26	1145
	N1P0K1	20.4	2.5	23.4	2.7	11.1	3.0	66	38	8	180	23	446
	N1P1K0	19.8	3.9	16.8	3.5	12.6	3.7	62	45	8	109	25	602
	N1P1K1	22.3	3.7	22.2	2.2	14.4	3.4	76	44	9	86	24	744
	N0P1K1	20.5	3.7	22.7	2.4	11.7	3.0	59	40	8	105	22	456
	N1P1K2org	21.7	3.0	23.9	1.9	13.0	2.8	75	45	9	44	24	752
Baldegg	N1P1K2org	22.1	4.4	31.6	2.0	8.2	2.1	38	20	7	23	28	747
	N1P1K2org/min	24.1	4.2	29.3	1.7	11.9	2.6	67	30	8	45	30	1246
	N2P2K1org I	28.3	5.1	30.9	5.4	9.0	2.6	46	31	7	25	30	356
	N2P2K1org II	25.1	6.1	20.4	4.7	11.5	2.5	60	29	7	19	33	968

Table A.3: Annual mean DRIS indices calculated for grasses using the norms and coefficients of variance from Bailey et al. (1997a).

Location	Treatment	DRIS - indices				
		N	K	P	Ca	Mg
Les Verrières	N1P0K0	6	-7	-4	-8	8
	N1P0.5K0.5	9	0	11	-19	-1
	N1P1K1	9	4	19	-23	-7
	N1P2K2	14	12	26	-33	-14
Watt	N0P0K0	6	-1	-2	-9	5
	N1P0K1	21	15	0	-33	0
	N1P1K0	2	-9	16	-23	10
	N1P1K1	10	7	18	-32	0
	N0P1K1	10	8	22	-34	-2
	N1P1K2org	16	14	5	-25	-5
Baldegg	N1P1K1org	18	15	25	-33	-22
	N1P1K1org/min	23	14	24	-44	-16
	N2P2K2org a	19	12	26	-33	-19
	N2P2K2org b	18	10	21	-28	-19

Additional results from DRIS and nutrient analysis

DRIS N indices were positive for all treatments except W-N1P1K0 (Table A.3). Its negative DRIS N might result from K deficiency which was reported to reduce N uptake by plants (Cushnahan et al., 1995; Minotti et al., 1968). Because W-N0P0K0 was not fertilized at all but had N concentrations (Table A.2) below 20 mg g^{-1} which is interpreted as N limited (Bailey et al., 1997a; Bélanger and Gastal, 2000) and showed a severe yield reduction, N limitation should be considered as well as co-limitation with other nutrients. However, the positive DRIS N index and negative DRIS P, K and Ca lead to the conclusion that in this treatment N was not the limiting nutrient despite the lack of N fertilization. For many other treatments we also found N concentrations below 20 mg N g^{-1}, but these were not reflected by DRIS N and had no effect on yield in the complete fertilized treatments (e.g. N1P1K1), thus they could only be considered as a latent N deficiency or a part of co-limitation with other nutrients (see below).

The DRIS K (Table A.3) index in V was always lower than the P index, indicating that K limitation was stronger than P limitation as it was hypothesized in chapter two. The positive DRIS P index for V-N1P0.5K0.5 indicates that P was not severely limited and thus the observed yield reduction is mainly caused by the K limitation. Consequently the K limitation should be relieved by fertilization.

The DRIS Ca indices were negative in all treatments and the Ca concentrations (Table A2) in Les Verrières and Watt were below the sufficient range of 4 to 8 mg g^{-1} from Whitehead (2000). Ca concentrations lower than 3.8 and 3.4 mg g^{-1} were also reported for perennial ryegrass and tall fescue from a non-Ca-limiting experiment by Sinclair et al. (2006). Loneragan and Snowball (1969) found no limitation of grass production above 0.7 mg Ca g^{-1}. Since the lowest Ca concentration in our investigation was 2.4 mg g^{-1} in W-N1P0K1, all treatments were well above this threshold and thus Ca can be considered as not limiting. That indicates that the published sufficient nutrient ranges (Table A.1) do not necessarily reflect critical values for plant growth.

The DRIS Mg index showed both negative and positive values. However, with the lowest observed Mg concentration of 1.3 mg g^{-1} the concentrations were within the sufficient range of 1 to 3 reported by Whitehead (2000). Concentrations of Ca and Mg may decline either because of competition with K or because of suboptimal growth conditions, e.g. drought. Thus, a negative sum of DRIS Ca and DRIS Mg may reflect the degree of limitation by non-nutritional constraints such as growth conditions or vegetation period if K deficiency is excluded. Bailey et al. (1997a; 1997b) suggested that DRIS Ca+Mg can be used as an internal standard other than yield when all other indices are positive. A negative DRIS Ca+Mg was observed in all treatments fertilized as recommended or above and confirms that growth was not limited by P, N, K, Ca and Mg nutrition. However, the relationship of Ca and Mg to the other nutrients in the DRIS calculation is not fully understood. Thus, DRIS Ca and DRIS Mg should be interpreted carefully.

For the following elements I will only present the results. For Sulphur (S) only N0P0K0 and N1P1K2org in W were found below 2 mg S g^{-1} which was identified as a critical value (Barker and Pilbeam, 2007; Whitehead, 2000). The values we found for Fe (24 to 70 mg kg^{-1}) were always below the sufficient ranges in literature (Table A.1). Although in the lower part, the observed concentrations for Zn, Cu and B were in the reported sufficient ranges from Whitehead (2000), respectively. For Mn we found low values in the intensively used grasslands in B, with an exceptionally low value in N2P2K1org II. This grassland showed a moist hydrology meaning a slow drying after rainy periods (personal observation). Reasons for that can be found in the landscape exposition (shadowed most of the day) and relatively high clay content in the soil.

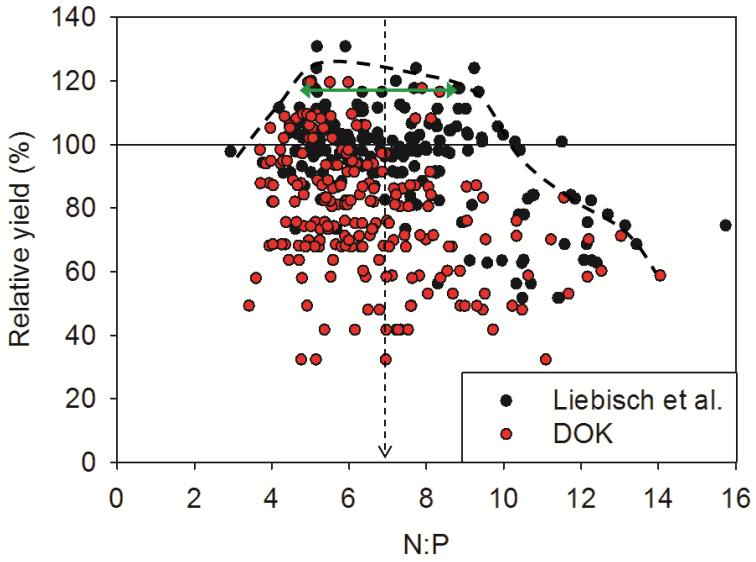

Figure A.2: Exemplary derivation of sufficient range and DRIS norm of N:P in grasses using a border line procedure (BOLIDES).The data from this study (black circles, chapter 2) were combined with data from another long term fertilizer experiment (DOK) in 2007 (red circles) from A. Oberson (personal communication). The horizontal line at 100% divides a high and low yielding sample population. This level is set arbitrarily and divides a low range of ratios (high yield) from a high range of ratios (low yield) as needed for norm derivation.

Additional information on soil analysis

Table A.4: Selected mean soil P indicators investigated in this thesis as affected by location and fertilizer treatment. The least significant difference (LSD) was calculated using an alpha of 0.05 for the three locations separately and for all locations together. The soil P indicators are P extracted with CO_2-saturated water (P_{CO2}), deionised water (P_{H2O}), anion exchange resin membranes (P_{res}), ammonium-acetate EDTA (P_{AAE}), ammonium-oxalate (P_{ox}), sulphuric acid before (P_i) and after incineration (P_{tot}). The isotopic exchange of ^{33}P within one minute (E_{1min}) and 30 days (E_{30days}) is shown. Additionally we measured microbial (P_{mic}) and organic P (P_{org}) and the degree of P saturation (DPS).

Location	treatment	P_{CO2} [a]	P_{H2O} [b]	P_{res} [c]	E_{1min} [b]	P_{AAE} [a]	E_{30days} [d]	P_{ox} [b]	P_i [e]	P_{tot} [e]	P_{mic} [c]	P_{org} [e]	DPS [f]
		mg kg^{-1}											g 100 g^{-1}
Les Verrières	N1P0K0	0.20	0.80	1.3	4.3	6.2	105	242	117	565	56	448	3.72
	N1P0.5K0.5	0.25	0.96	7.0	4.5	28.7	156	271	191	688	55	498	4.24
	N1P1K1	0.39	1.20	15.3	4.9	28.6	185	304	254	733	51	478	5.11
	N1P2K2	0.55	2.13	33.4	6.5	43.1	220	384	350	842	39	491	7.33
LSD		0.17	0.78	7.5	1.7	23.4	66	111	89	122	10	56	2
Watt	N0P0K0	0.37	0.90	1.1	2.7	4.4	59	168	107	581	34	474	8.81
	N1P0K1	0.34	0.76	1.0	2.1	4.9	52	195	68	692	33	624	9.53
	N1P1K0	1.26	4.22	9.6	9.7	19.4	73	223	164	679	41	516	13.91
	N1P1K1	0.84	2.30	7.4	4.9	12.0	63	227	163	659	40	496	13.69
	N0P1K1	0.86	2.47	7.0	5.3	11.8	76	219	155	731	37	576	12.65
	N1P1K2org	0.96	3.03	1.1	7.6	7.1	75	192	179	713	52	534	12.25
LSD		0.71	1.58	4.9	3.6	6.9	41	89	165	323	12	348	5
Baldegg	N1P1K2org	4.60	12.01	11.3	17.1	55.3	136	403	538	1574	74	1036	43.56
	N1P1K2org/min	1.77	6.70	11.8	12.5	31.3	169	471	602	1610	54	1008	26.45
	N2P2K1org I	16.70	38.20	58.6	47.5	238.5	212	743	1100	2104	51	1004	73.14
	N2P2K1org II	10.70	41.75	54.5	57.0	239.3	276	840	1439	2506	57	1067	85.12
LSD		10.03	6.34	9.3	25.2	88.4	69	91	307	308	9	204	28
LSD total	a=0.05	6.77	4.44	8.6	17.2	61.5	68	112	244	350	12	284	14

[a b c] methods were described in previous chapters: [a] = chapter four, [b] = chapter three, [c] =chapter five,

[d] E_{30days} was calculated according to fardeau et al. (1985) with r_i/R calculated for 30 days month (43200 minutes) and P_i,

[e] extraction with 0.5 M H_2SO_4 before (P_i) and after incineration at 550°C for 8 hours (P_{tot}), $P_{org} = P_{tot}-P_i$,

[f] DPS = $(P_{ox}/\alpha(Al_{ox}+Fe_{ox}))*100$ (Schoumans, 2000). The factor α was calculated as $1-r_1/R$ from Table A.7.

Table A.5: Correlation coefficients (ρ) of selected soil P with plant yield and plant P indicators calculated by the Spearman's rank correlation. Levels of significance for absolute values: $\rho > 0.42$ p= 0.001, $\rho > 0.37$ p= 0.01, $\rho > 0.27$ p=0.05. Soil P indicators are described in detail in Table A.4. Plant indicators are yield, relative yield, P concentration, P uptake, the P nutrition index (PNI), the N:P ratio and the P index derived from the diagnosis recommendation integrated system (DRIS P). The derivation or calculation of the plant indicators is described in detail in chapter two.

P indicator	Yield ($t\ ha^{-1}yr^{-1}$)	Relative yield (%)	P-concentration ($mg\ g^{-1}$)	P uptake ($kg\ ha^{-1}yr^{-1}$)	PNI	N:P	DRIS P
P_{CO2}	0.87	0.5	0.79	0.85	0.7	-0.41	0.59
P_{H2O}	0.85	0.54	0.84	0.87	0.75	-0.44	0.65
P_{res}	0.57	0.52	0.86	0.77	0.83	-0.67	0.76
P_{mic}	0.39	0.34	0.4	0.37	0.29	0.05	0.14
P_{AAE}	0.63	0.53	0.86	0.77	0.83	-0.57	0.74
P_{ox}	0.61	0.47	0.82	0.74	0.74	-0.42	0.65
P_{min}	0.7	0.57	0.82	0.79	0.74	-0.42	0.63
P_{org}	0.76	0.37	0.71	0.73	0.6	-0.28	0.51
P_{tot}	0.74	0.53	0.84	0.81	0.77	-0.43	0.66
E_{1min}	0.75	0.49	0.77	0.78	0.69	-0.39	0.6
E_{30days}	0.41	0.45	0.65	0.55	0.62	0.4	0.53
DPS	0.82	0.39	0.68	0.76	0.56	-0.28	0.48

Table A.6: Coefficients of correlation (ρ) between selected soil P indicators calculated by the Pearson product-moment correlation. Level of significance: $\rho > 0.42$ $p = 0.001$. Soil P indicators are described in detail in Table A.4.

P indicator	P_{CO2}	P_{H2O}	P_{res}	P_{AAE}	P_{ox} (mg kg^{-1})	P_i	P_{tot}	E_{1min}	$E_{30\ days}$	DPS (g 100 g^{-1})
P_{CO2}	1									
C_P	0.86	1								
P_{res}	0.79	0.88	1							
P_{AAE}	0.91	0.96	0.91	1						
P_{ox}	0.80	0.92	0.91	0.91	1					
P_i	0.82	0.94	0.87	0.93	0.96	1				
P_{tot}	0.79	0.90	0.79	0.87	0.95	0.96	1			
E_{1min}	0.78	0.97	0.83	0.9	0.89	0.91	0.87	1		
E_{30days}	0.54	0.65	0.78	0.71	0.81	0.79	0.71	0.68	1	
DPS	0.80	0.94	0.71	0.87	0.86	0.89	0.91	0.90	0.49	1

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Table A.7: Selected parameters derived from ^{33}P exchange kinetics. See the description of the method in chapter three (3.3.5.). E_{30days} was calculated according to Fardeau et al. (1985) using r_t/R calculated for 30 days (43200 minutes) and P_i (Table A.4).

Location	Treatment	n	r_1/R	E_{1min}	E_{30days}
				(mg kg^{-1})	
Les Verrières	N1P0K0	0.52	0.19	4	105
	N1P0.5K0.5	0.49	0.21	4	156
	N1P1K1	0.47	0.25	5	185
	N1P2K2	0.42	0.33	6	220
Watt	N0P0K0	0.39	0.38	3	59
	N1P0K1	0.45	0.36	2	52
	N1P1K0	0.25	0.45	10	73
	N1P1K1	0.32	0.47	5	63
	N0P1K1	0.32	0.46	5	76
	N1P1K2org	0.27	0.40	8	75
Baldegg	N1P1K2org	0.21	0.66	17	136
	N1P1K2org/min	0.28	0.55	13	169
	N2P2K1org a	0.16	0.80	48	212
	N2P2K1org b	0.16	0.70	57	276

Table A.8: Mean aluminum (Al_{ox}), phosphorus (P_{ox}) and iron (Fe_{ox}) extracted with ammonium oxalate and the calculated degree of P saturation (DPS). $DPS = (P_{ox}/0.5(Al_{ox}+Fe_{ox}))*100$ (Schoumans, 2000). For DPS_{adj} 0.5 was replaced by the value for $1-r_1/R$ calculated from Table A.7.

Location	Treatment	Al_{ox}	P_{ox}	Fe_{ox}	DPS	DPS_{adj}
			(mg kg^{-1})		(g 100 g^{-1})	
Les Verrières	N1P0K0	1443	242	6591	6	4
	N1P0.5K0.5	1522	271	6581	7	4
	N1P1K1	1437	304	6494	8	5
	N1P2K2	1489	384	6416	10	7
Watt	N0P0K0	835	168	2301	11	9
	N1P0K1	889	195	2396	12	10
	N1P1K0	811	223	2084	15	14
	N1P1K1	870	227	2229	15	14
	N0P1K1	891	219	2386	13	13
	N1P1K2org	775	192	1909	15	12
Baldegg	N1P1K2org	890	403	1945	28	44
	N1P1K2org/min	1084	471	3197	22	26
	N2P2K1org a	985	743	2353	46	73
	N2P2K1org b	901	840	1747	64	85

Table A. 9: Mean and standard deviation (sd) of P extracted with CO_2 saturated water (P_{CO2}) and ammonium-acetate EDTA (P_{AAE}) as affected by location, treatment and soil sampling depth.

Location	Treatment	P_{CO2} (mg kg^{-1})							
		0-5		5-10		10-20		20-40	
		mean	sd	mean	sd	mean	sd	mean	sd
Les Verièrres	N1P0K0	0.20	0.04	0.21	0.12	0.16	0.23	0.01	0.01
	N1P0.5K0.5	0.25	0.02	0.19	0.15	0.04	0.01	0.01	0.01
	N1P1K1	0.39	0.07	0.17	0.02	0.05	0.02	0.03	0.01
	N1P2K2	0.55	0.13	0.22	0.06	0.07	0.02	0.02	0.02
Watt	N0P0K0	0.37	0.07	0.17	0.04	0.11	0.01	0.01	0.00
	N1P0K1	0.34	0.03	0.18	0.05	0.10	0.03	0.04	0.06
	N1P1K0	1.26	0.38	0.36	0.17	0.16	0.05	0.01	0.01
	N1P1K1	0.84	0.42	0.29	0.10	0.16	0.02	0.01	0.00
	N0P1K1	0.86	0.30	0.29	0.14	0.15	0.04	0.00	0.01
	N1P1K2org	0.96	0.34	0.46	0.09	0.19	0.04	0.02	0.02
Baldegg	N1P1K2org	4.6	1.03	2.2	0.24	1.0	0.18	0.6	0.04
	N1P1K2org/min	1.8	0.48	0.7	0.16	0.4	0.17	0.0	0.02
	N2P2K1org a	16.7	5.13	10.3	3.00	5.4	1.44	1.1	0.30
	N2P2K1org b	10.7	8.21	9.8	7.89	8.1	6.87	10.9	7.94
		P_{AAE} (mg kg^{-1})							
Les Verièrres	N1P0K0	6.16	1.20	3.51	0.54	1.82	0.23	1.10	0.31
	N1P0.5K0.5	28.74	18.37	4.87	1.37	2.54	0.72	1.45	0.39
	N1P1K1	28.63	8.75	8.74	2.65	3.51	1.29	1.49	0.98
	N1P2K2	43.15	4.83	13.65	2.26	4.30	1.22	1.91	0.62
Watt	N0P0K0	4.37	0.86	3.37	0.90	2.42	0.83	1.01	0.41
	N1P0K1	4.88	0.93	2.67	0.47	1.75	0.58	0.89	0.29
	N1P1K0	19.44	5.06	4.99	0.93	3.52	1.74	1.44	0.61
	N1P1K1	11.98	4.57	4.63	0.88	3.16	1.16	1.25	0.30
	N0P1K1	11.79	1.28	4.81	1.09	3.70	1.89	1.20	0.44
	N1P1K2org	7.14	0.25	4.83	1.11	3.52	0.72	1.67	0.26
Baldegg	N1P1K2org	55.3	4.96	36.5	5.61	20.0	2.51	8.8	3.79
	N1P1K2org/min	31.3	6.60	18.9	3.04	14.1	3.88	5.4	2.37
	N2P2K1org a	238.5	73.41	195.4	62.80	137.0	33.40	49.7	17.43
	N2P2K1org b	239.3	43.82	189.1	29.80	124.8	20.49	46.3	11.38

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Curriculum vitae

Frank Liebisch, Dipl-Ing agr.

Born 27th of June 1978 in Gera (Germany)

04/2007- 04/2011	PhD student/ scientific collaborator at ETH Zurich, Switzerland, Institute of Agricultural Sciences, Group of Plant Nutrition (Prof. E. Frossard).
2001-2006	Graduate studies in Horticulture at the Leibniz University Hanover, Germany, Diploma in Agricultural Sciences Highlights during that time: Employed in different institutes as research associate or educational helper. Internship and diploma thesis within the project: Protected cultivation - an approach to sustainable vegetable production in the humid tropics (DFG-Research Group FOR 431) at the Asian Institute of Technology (AIT), Thailand
1999-2001	Apprenticeship as Gardener for ornamental crops (Parks and gardens department, Jena).
1998-1999	Compulsory community service at the Office for Environment in Eisenberg (Germany).
1997	Abitur (German university entrance qualification).