Seasonal dynamics and turnover of microbial phosphorusin a permanent grassland

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ORIGINAL PAPER

Seasonal dynamics and turnover of microbial phosphorus in a permanent grassland

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Abstract Microbial activity is known to be high under permanent grassland, but consequences for soil phosphorus (P) dynamics and availability are not well understood. Our main objective was to assess the microbial P turnover derived from the seasonal fluctuations in microbial P (measured as hexanol-labile P (P_{hex}) at 13 sampling times during 9 months) in a permanent grassland in Switzerland as affected by different P fertilization treatments (P inputs of 0 (NK) or 17 kg P ha^{-1} $year^{-1}$ in the form of superphosphate (NPK) or dairy slurry (DS)). Plant P uptake, available inorganic P measured as resin-extractable P (P_{res}), potential organic P mineralization indicated by acid phosphomonoesterase activity and climatic conditions were also recorded. Despite significant differences in plant P uptake and P_{res} (NPK>DS>NK), the turnover rate of P_{hex} was similar in all treatments (approximately once per growing season). Thus, the seasonal P flux through P_{hex} was similar to the stock of P_{hex}, which was about 18, 25 and 37 kg P ha^{-1} in NK, NPK and DS, respectively, and larger than the corresponding seasonal plant P uptake of 6, 17 and 12 kg P ha^{-1}. The estimate of P_{hex} turnover based on seasonal dynamics did not confirm previous tracer-based findings of a much faster P_{hex} turnover under low availability of inorganic P, and the magnitude of P_{hex} turnover depended on the number of sampling points taken into account. Fluctuations in P_{res} and P_{hex} were related to soil moisture and indicated competition between plants and microorganisms for available P.

Keywords Phosphorus · Microbial biomass · Turnover · Seasonal fluctuations · Permanent grassland

Introduction

Soils under grassland typically show higher contents of soil organic matter than arable soils, especially in the absence of ploughing (Whitehead 1995). Accordingly, organic forms of phosphorus (P) are usually more prominent in grassland soils. In 29 temperate grassland soils, on average, 53 % (±11 %) of total P was in organic and condensed forms (Turner et al. 2003b), compared to 11 % (±6 %) in 18 arable soils (Turner et al. 2003a). Likewise, the proportion of total P held in the microbial biomass is typically greater in permanent grassland than in arable soils (Oberson and Joner 2005). As a result, the microbial processes of organic P mineralization, P immobilization into the living microbial biomass and release upon cell death (i.e. remineralization) can be expected to play an important role in P dynamics of grassland soils, with potential effects on plant P nutrition (Simpson et al. 2012).

These microbial fluxes can be quantified under controlled laboratory conditions, where the use of isotopic dilution techniques permits to derive net organic P mineralization from the difference of gross P mineralization and microbial immobilization (Bünemann et al. 2007). Such experiments have been conducted under steady state conditions, i.e. under constant respiration rates and microbial biomass, and have rendered basal gross P mineralization rates in the order of 1.0–2.5 mg P kg^{-1} day^{-1} soil for arable and forest soils (Achat et al. 2009; Oehl et al. 2001, 2004). Higher gross P

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mineralization rates of 3.1 and 8.2 mg P kg^{-1} day^{-1} have been measured under permanent grassland with and without mineral P fertilization, respectively (Bünemann et al. 2012). The main component of these rates was microbial P immobilization, which was very fast and extensive, especially under P limitation.

Complete renewal of the microbial P pool in isotopically (^{32}P or ^{33}P) labeled soils is reached when the specific activities ($^{32}P/^{31}P$ or $^{33}P/^{31}P$) in water-extractable and microbial P converge (Oberson and Joner 2005), or in other words, when the newly immobilized P has entirely replaced the P initially present in the microbial biomass. Such tracer-based estimates of microbial P turnover times at steady state range between 70 and 160 days for the surface horizon of non-amended arable and forest soils (Achat et al. 2010a; Oberson and Joner 2005). In the grassland studied by Bünemann et al. (2012), the tracer-derived microbial P turnover time was about 90 days with P fertilization, but only 5 days without.

In the field, microbial processes in P cycling are affected by fluctuations in soil moisture and temperature (Blackwell et al. 2010), competition between plants and microorganisms for P (Rousk et al. 2007), interactions between microbial biomass and grazers such as amoebae, protozoa and nematodes (Bloem et al. 1997; Cole et al. 1978), and inputs of organic matter from above- and below-ground plant residues (Chen et al. 2003). As a result, net changes in microbial P throughout the year have been observed. For example, when measured with relatively high temporal resolution (26 sampling points during 2 years) in a grazed pasture in New Zealand, microbial P varied between 80 and 130 mg P kg^{-1} soil (Perrott et al. 1990). Fluctuations in microbial P as well as acid phosphomonoesterase activity were positively related to soil moisture and not affected by mineral fertilizer additions. Although fluctuations in microbial P were smaller (73 to 93 mg P kg^{-1}) at another pasture site in New Zealand with lower P availability, decreases in microbial P between sampling points were generally larger than plant P uptake in unfertilized plots during the corresponding period of time (Perrott et al. 1992). Thus, Perrott et al. (1992) concluded that the P released from the microbial biomass could potentially account for pasture P uptake during the same period.

Such decreases in microbial biomass can be used to derive an estimate of microbial turnover rate by dividing the sum of losses by the average microbial biomass (McGill et al. 1986). Since this does not capture concurrent growth and death, it is a minimum estimate of microbial turnover which would need to be verified by tracer data. McGill et al. (1986) calculated turnover rates of 0.2–4 $year^{-1}$ for microbial C in various arable crop rotations in Canada. Similar calculations for arable fields in Germany showed a faster turnover rate for microbial N (0.9–1.1 $year^{-1}$) than for microbial C (0.3–1.2 $year^{-1}$) in three out of four experimental fields (von Lützow and Ottow 1994). For microbial P, the concept was applied to arable soils in Switzerland, rendering turnover rates between 0.3 and 0.6 $year^{-1}$ (Oberson et al. 1995), and to grassland and forest soils in New Zealand, where faster turnover rates of 0.8–1.3 $year^{-1}$ were found (Chen et al. 2003).

Our main objective was to assess the effect of different fertilization regimes in a permanent grassland on microbial P turnover derived from the seasonal dynamics of microbial P (measured as hexanol-labile P) during one growing season. We hypothesized that microbial P turnover would be faster in the absence of P fertilization, as previously indicated under laboratory conditions (Bünemann et al. 2012). To reveal a potential competition between plants and microorganisms, we also assessed plant P uptake, fluctuations in rapidly available inorganic P (measured as resin-extractable P) and acid phosphomonoesterase activity as an indicator of potential organic P mineralization. Climatic conditions (soil and air temperature, precipitation and soil moisture) were monitored to identify the factors underlying the seasonal dynamics of the P pools.

Material and methods

Experimental site, management and treatments

The two adjacent fertilizer experiments we studied here have a randomized block design and a plot size of 2 m × 5 m (Fig. 1, supplementary electronic material). They are located near Watt in the Swiss lowland (47°26′44″N, 8°29′32″E, 500 m above sea level) and were started in 1992 by the Federal Research Station Agroscope Reckenholz-Tänikon (ART) on a permanent grassland (Huguenin-Elie et al. 2006). Annual temperature and precipitation between 1999 and 2009 averaged 9.8 °C and 1,077 mm, respectively. The mesic grassland grows on a medium-deep (50–70 cm) Cambisol. Soil texture in the top 10 cm measured at the beginning of the experiment was 220, 340 and 440 g kg^{-1} of clay, silt and sand, respectively (Philipp et al. 2004). The vegetation is an *Arrhenatherion elatioris* association with about 30 angiosperm species (Liebisch et al. 2013). It is managed at low intensity, with three harvests per year and a late first harvest (after June 15) to preserve plant diversity. In 2009, harvests took place on days of the year 167, 216 and 267.

Three fertilizer treatments were chosen for this study (Table 1). The two mineral fertilized treatments from experiment 1 received N and K (NK) or N, P and K (NPK), while the organically fertilized treatment (DS) from experiment 2 received dairy slurry. Mineral N was applied as ammonium nitrate (split into three applications: at the beginning of the growing season, after the first and after the second harvest), and mineral P and K as superphosphate and potassium chloride, respectively (both applied at the beginning of the season, Fig. 1). The

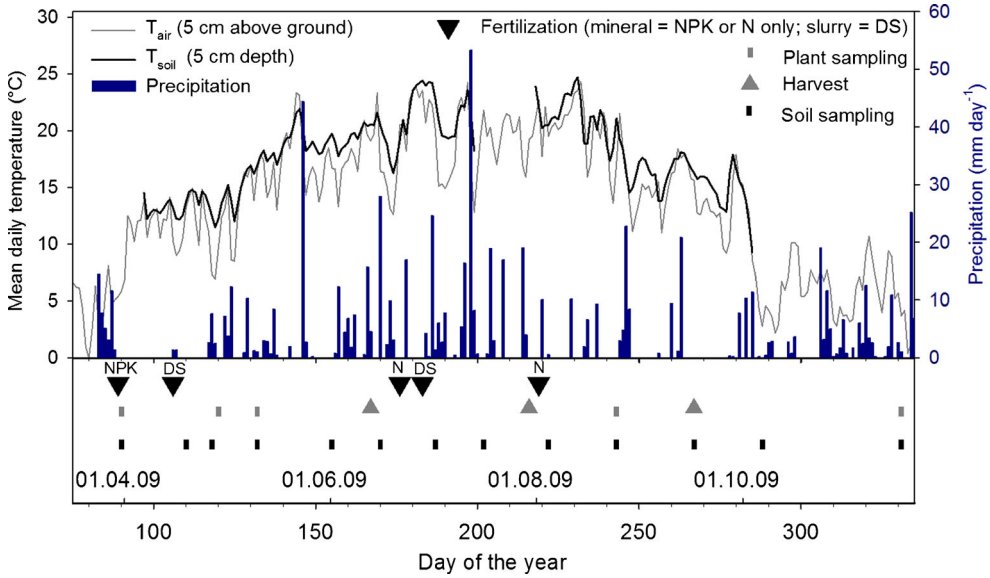

Fig. 1 Daily air and soil temperature, daily precipitation, experimental management (fertilizer additions in the form of NPK, N only, or dairy slurry (DS)) and harvest and sampling dates. Aboveground temperature and precipitation were measured by a meteorological station in 2-km distance to the field. The soil temperature was measured at the investigated field site (with interruptions due to technical problems)

organically fertilized treatment received an annual application of 24 m^3 ha^{-1} dairy slurry, which was split into two equal applications at the beginning of sward growth and after the first harvest. The slurry was diluted with water (1:1) for spreading. Amounts of nutrients applied are shown in Table 1. The number of species and the dominant species were similar in all the treatments (Liebisch et al. 2013).

Table 1 Fertilization type, amounts of annually applied nutrients and selected soil properties (in the top 5 cm) in 2009 in the three fertilization treatments NK, NPK and DS in a mesic grassland managed at low intensity since 1992

		NK	NPK	DS
Fertilization				
Type		Mineral	Mineral	Dairy slurry
N	kg ha^{-1} $year^{-1}$	45	45	55[a]
P	kg ha^{-1} $year^{-1}$	0	17	17[a]
K	kg ha^{-1} $year^{-1}$	83	83	102[a]
Soil properties				
pH[b]		5.2±0.1	5.4±0.0	6.8±0.1
Total C[c]	g kg^{-1}	24.7±0.5	24.9±1.2	29.8±1.0
Total N[c]	g kg^{-1}	3.0±0.1	3.1±0.2	3.8±0.1
Total P[d]	mg kg^{-1}	664±3.1	784±12.7	834±6.4
Total organic P[e]	mg kg^{-1}	366±26.0	439±21.0	480±5.2

Means ± standard deviations (n = 4)

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

[b] Soil/water ratio = 1:2.5

[c] Measured by CN analyser (Flash EA, Thermo Electron Corporation)

[d] Extraction with 0.5 M H_2SO_4 after incineration

[e] According to Saunders and Williams (1955)

Climatic data

On site, a data logger (HOBO Micro Station, Onset Computer Corporation, Bourne, MA, USA) was implemented outside the experimental plots to measure the soil temperature (T_{soil}) at 5-cm depth. Additional environmental data (daily precipitation and average air temperature (T_{air}) at 5 cm above the ground) were obtained from the closest meteorological station (Zurich Affoltern, 47°26′N, 8°31′E, 443 m above sea level, 2-km distance to the field), as available from MeteoSwiss (IDAWEB 2009).

Soil sampling

The soils were sampled 13 times throughout the season from March to November 2009 (Fig. 1). The sampling period covered a total of 241 days. The sampling dates were chosen to capture the effects of fertilization, harvests and environmental conditions such as dry spells, precipitation events and temperature changes. At each sampling, three randomly distributed soil cores of 2-cm diameter and 5-cm depth were taken in each plot, excluding 0.5 m from each border, made into one composite sample and stored at 4 °C in the dark. Within 2 days, the samples were sieved through a 2-mm mesh to remove visible plant material and stones, and gravimetric water content was determined. Extractions for resin-extractable and hexanol-labile P took place within 4 days after sampling. The remaining soil was frozen at −20 °C for later determination of acid phosphomonoesterase activity. Soil pH, soil organic C, total N and total and organic P were determined on air-dried soil taken on day 243 of the year.

The soil pH (Table 1) was significantly higher in DS than those in the other two treatments due to a pH gradient caused by the topography, with the organic fertilizer trial located

towards the top of a slight slope. The soil organic C and total N were also elevated in DS compared to those in NK and NPK, most probably for the same reason, while higher total P in NPK and DS than that in NK was likely to result from P inputs since 1992.

Resin-extractable and hexanol-labile P

Resin-extractable P (P_{res}) and hexanol-labile P (P_{hex}) were determined in duplicates by extraction with anion exchange resin membranes and simultaneous hexanol fumigation (Kouno et al. 1995). In detail, 3-g moist soil was shaken with 30 ml of double-distilled water and two resin strips of 3 cm× 2 cm (BDH Laboratory Supplies #55164 2S, Poole, England) for 16 h at 150 rpm on a horizontal shaker, with addition of 1 ml hexanol to fumigated subsamples. The membranes were rinsed with water, and P was eluted with 0.1 M NaCl/HCl, followed by colorimetric determination of P concentrations in the eluates with malachite green (Ohno and Zibilske 1991).

The concentration of P in non-fumigated subsamples (P_{res}) was subtracted from that in the fumigated subsamples to render P_{hex}. Hexanol-labile P was not corrected for incomplete recovery of P due to sorption, since the mean recovery of a known addition of P as orthophosphate to a similar set of soil samples taken prior to this experiment was high (93 %) and similar across treatments. We did not apply a conversion factor (kp) either because it varies between soils and methods (Oberson and Joner 2005) and has not been determined for the soil and protocol used in this study. We included a dry standard soil (unfertilized soil from the same site) on each of the 13 extraction dates. Based on the low variation (mean±standard deviation) of P_{hex} (7.9±0.9 mg kg^{-1}) and P_{res} (10.1± 0.5 mg kg^{-1}) in this standard soil, analytical variation between extraction dates was considered to be negligible.

Acid phosphomonoesterase activity

On eight sampling dates, acid phosphomonoesterase activity was measured in a microplate design following the method of Marx et al. (2001) as modified by Poll et al. (2006). Briefly, 1 g of frozen soil was dispersed in 100 ml of autoclaved H_2O using an ultrasonic probe. The suspension was transferred to a microplate with six analytical replicates, 4-methylumbelliferyl phosphate was used as substrate, and the assay was buffered with 0.1 M MES buffer at pH 6.1. The linear increase in fluorescence was measured at five time points during 180 min (FLx800, Biotek) and converted into a rate of phosphate release in μmol g^{-1} h^{-1} based on standard curves with additions of 4-methylumbelliferon to each soil. At pH 6.1, the vast majority of the measured activity can be attributed to acid rather than to alkaline phosphomonoesterases (Nannipieri et al. 2011).

Plant sampling and analysis

Plant sampling in all treatments took place at the beginning of the experiment (day 90) and at each of the three harvests (Fig. 1). In NPK only, the plants were also sampled on four additional dates to gain a better resolution of plant growth. The plants were cut at 4 cm above the ground using electric scissors on a randomly selected area of 0.5 m×0.5 m with at least 0.5-m distance to the plot border. The different spots were sampled throughout the year, which limited the possible number of plant samplings.

The plant samples were dried at 60 °C for 3 days before dry matter determination. The subsamples of the milled plant material from each of the three harvests were analysed for P concentration by incineration at 550 °C for 8 h, solubilization of the ashes in 15 M nitric acid and colorimetric P determination using malachite green.

Calculation of P_{hex} turnover rate and flux

The concept of McGill et al. (1986) to estimate the microbial C turnover from seasonal fluctuations in microbial C was applied to calculate the turnover rate of P_{hex} (Eq. 1):

$$P_{hex}\,\text{turnover rate} = \frac{\sum P_{hex}\text{losses}}{\text{mean}P_{hex}}, \quad (1)$$

with the sum of P_{hex} losses ($\sum P_{hex}$losses) calculated from the sum of decreases in P_{hex} between two subsequent measurements, and the meanP_{hex} is defined as the average P_{hex} of all the measurement dates, both given in mg P kg^{-1} of dry soil. In this study, the P_{hex} turnover rate is expressed per season and accounts for the period of 241 days between the first and the last sampling. Accordingly, the P_{hex} turnover time (in days) was derived by dividing the turnover rate by the number of days per season (241 days).

The P flux through the microbial biomass (P_{hex} flux) was then calculated as shown in Eq. 2:

$$P_{hex}\text{flux} = \frac{\text{mean}P_{hex} \times \rho \times D \times \text{area}}{P_{hex}\text{turnover rate}}, \quad (2)$$

where ρ is the bulk density of the topsoil (1.05 g cm^{-3}), D is the sampling depth of 5 cm and the area is 1 ha. The resulting numerator of Eq. 2 is the mean P_{hex} stock (in kg P ha^{-1}), and the P_{hex} flux for the period of 241 days is thus given in kg P ha^{-1}. The mean P_{res} stock (in kg P ha^{-1}) was calculated similar to the mean P_{hex} stock.

Statistical analysis

Statistical analyses were performed with R version 2.8.1 (R Development Core Team 2008). Prior to analysis, the percentage data of soil moisture were transformed to their arc sin.

Treatment and temporal differences within each treatment were analysed by ANOVA. If the F test was significant at $p<0.01$, the least significant difference (LSD) was calculated with an alpha of 0.05. The Holm adjustment for p values was always applied to control for false positives in multiple comparisons. Correlation coefficients (ρ) and their level of significance were calculated by the Pearson product–moment correlation.

Results

Climatic conditions

In 2009, there were 246 vegetation days (T_{air}>5 °C), with a continuous period between days 85 and 287 (Fig. 1). Longer continuous periods with T_{air} mostly above 20 °C were observed between days 179–186 and days 218–240, with daily maxima of 24.4 and 24.8 °C on days 183 and 231, respectively. The T_{soil} generally followed T_{air}, but with smaller fluctuations. Precipitation was reasonably well distributed throughout the season, averaging 85 mm (±46) per month. Only April (days 91–120) was exceptionally dry (15 mm). The changes in soil moisture (Fig. 2) reflected the distribution of precipitation, with similar fluctuations between the treatments. However, DS showed a higher water holding capacity of about 0.5 g g^{-1} compared to 0.45 g g^{-1} in the other two treatments. Soil moisture below 0.2 g g^{-1} was measured on days 155 and 243 by gravimetry. A soil moisture sensor outside the experimental plots additionally registered a period with soil moisture contents below 0.3 g g^{-1} between days 183 and 186 (Fig. 2, supplementary electronic material).

Seasonal dynamics of P_{res} and P_{hex}

The seasonal pattern of fluctuations in P_{res} was similar between the treatments, but at different levels (Fig. 2). The values ranged consistently in the order NPK≥DS≥NK. The amplitude of P_{res} was also largest in NPK, with average P_{res} varying between 6.5 mg P kg^{-1} on day 90 and 17.9 mg P kg^{-1} on day 155. Consistent maxima of P_{res} were observed at days 110, 155, 187, 243 and 288, partly coinciding with dry periods shortly before or at the sampling date.

Fluctuations in P_{hex} were not as synchronous between the treatments as for P_{res}, and the treatments ranged in a different order: DS≥NPK≥NK (Fig. 2). The minima of P_{hex} were observed on days 118, 155 and 267 in DS, on days 90, 118, 187 and 267 in NPK and on days 155 and 243 in NK. Simultaneous increases of P_{hex} in the three treatments were observed after days 155 and 267.

Acid phosphomonoesterase activity

Acid phosphomonoesterase activity showed no consistent change in response to the low soil moisture on days 155 and 243 (Fig. 2). In contrast to P_{res} and P_{hex}, the highest acid phosphomonoesterase activities were found in NK and lowest in DS, while NPK was intermediate (except on day 155).

Correlations between soil parameters measured throughout the season

Across all treatments, P_{hex} was positively correlated to soil moisture, while the correlation was negative for P_{res} (Table 2). Similar and partly significant correlations were found when each treatment was analysed separately. The acid phosphomonoesterase activity was negatively correlated to P_{res}, P_{hex} and soil moisture when all the treatments were considered, but these correlations were not significant within single treatments.

Plant biomass and P uptake

Harvested plant biomass declined from the first harvest to the subsequent ones (Fig. 3). The treatments ranged in the order NPK≥DS≥NK, with cumulative plant dry matter harvested in 2009 of 7.5, 6.2 and 4.7 t ha^{-1}, respectively. Total seasonal plant P uptake reflected the same order of treatments as the biomass, but the differences were even more pronounced (Table 3).

P_{hex} turnover rate and flux

The mean seasonal P_{hex} ranged in the order NK<NPK<DS (Table 3). In all the treatments, the sum of P_{hex} losses was similar to the mean P_{hex}. Consequently, P_{hex} was turned over once during the season, with mean turnover times between 224 and 293 days. The resulting P_{hex} flux was lowest in NK (18.1 kg ha^{-1} $season^{-1}$) and highest in DS (36.9 kg P ha^{-1} $season^{-1}$). Both P_{hex} flux and mean P_{hex} stocks were higher than seasonal plant P uptake, while mean P_{res} stocks were lower.

Discussion

Seasonal dynamics of P_{hex} and P_{res}

In a meta-analysis of seasonal fluctuations in soil microbial C and N, Wardle (1998) calculated the coefficient of variation (CV) over time in a given treatment as a measure of temporal variability. This allows comparing the extent of seasonal fluctuations between studies, regardless of the use of a correction factor for incomplete extraction of microbial biomass after

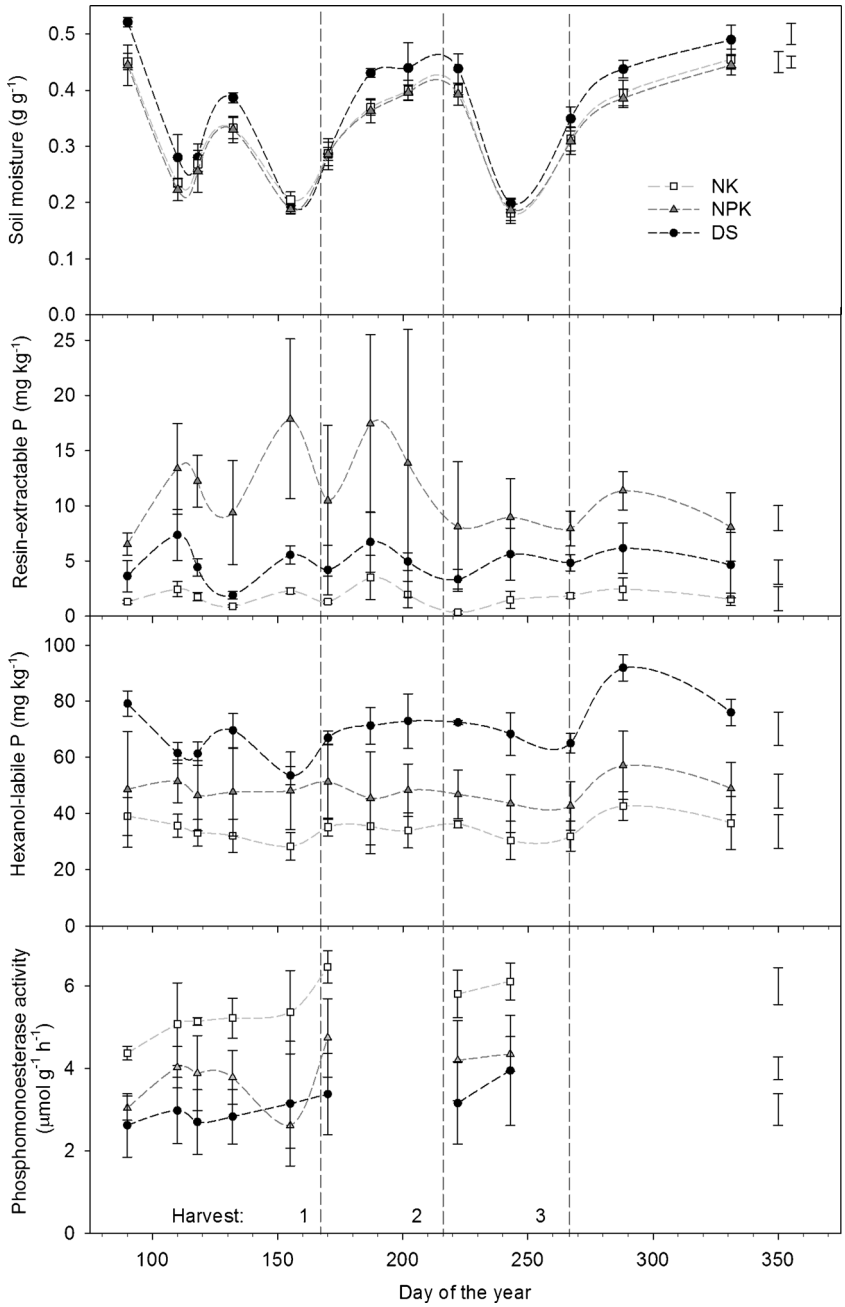

Fig. 2 Gravimetric soil moisture, resin-extractable P, hexanol-labile P and acid phosphomonoesterase activity in the three fertilizer treatments NK, NPK and DS in 2009. Means ± standard deviations ($n=4$). *Error bars* shown on day 350 indicate least significant difference (LSD test at $\alpha=0.05$, with Holm adjusted p values) for each treatment separately. *Vertical dashed grey lines* indicate the harvest dates

fumigation. Fluctuations in P_{hex} in our study (CV 8–13 %) were in the same range as those found for microbial P by Perrott et al. (1990) in highly productive pastures in New Zealand (CV 10–14 %) and by He et al. (1997) in fertilized pastures in the UK (CV 13–14 %), while Chen et al. (2003) observed greater temporal variation in microbial P (CV 31 %) in an unfertilized grassland in New Zealand. In all these studies, microbial P decreased during the periods with relatively low soil moisture content.

A positive relationship between P_{hex} and soil moisture content was also observed in our study (Table 2). In a previous paper (Bünemann et al. 2013), we conducted laboratory experiments on the effects of drying and rewetting on P dynamics, using non-fertilized soil from the same site as in the present study. A significant decrease in P_{hex} was observed when soils were dried to gravimetric water contents of 0.10 g g^{-1} or lower, whereas P_{hex} remained unchanged between water contents of 0.15 and 0.40 g g^{-1}. In the field, however, the lowest observed gravimetric water content was 0.18–0.21 g g^{-1} on days 155 and 243, and the minima of P_{hex} were not consistently found on these dates (Fig. 2). Therefore, the observed positive relationship between P_{hex} and soil moisture cannot be attributed to drying and rewetting effects but must have other underlying mechanisms such as the

Table 2 Coefficients of correlation between P_{res}, P_{hex}, acid phosphomonoesterase (PME) activity and soil moisture for the three fertilization treatments NK, NPK and DS alone or together

Treatment	Parameter	P_{res} mg kg^{-1}	P_{hex} mg kg^{-1}	Acid PME activity µmol g^{-1} h^{-1}	Soil moisture g g^{-1}
All					
	P_{res}	1			
	P_{hex}	0.09	1		
	Acid PME activity	−0.30**	−0.60***	1	
	Soil moisture	−0.17*	0.39***	−0.22*	1
NK					
	P_{res}	1			
	P_{hex}	−0.12	1		
	Acid PME activity	−0.06	0.11	1	
	Soil moisture	−0.16	0.49***	−0.29	1
NPK					
	P_{res}	1			
	P_{hex}	−0.10	1		
	Acid PME activity	−0.12	−0.21	1	
	Soil moisture	−0.29*	0.18	−0.06	1
DS					
	P_{res}	1			
	P_{hex}	−0.0	1		
	Acid PME activity	0.31	0.17	1	
	Soil moisture	−0.20	0.68***	−0.32	1

*p <0.05; **p <0.01; ***p <0.001

interaction between plants and microorganisms, which may be affected by changes in quantities and diffusion of root exudates.

Indeed, the large synchronous increase in P_{hex} in all fertilization treatments after the third harvest could be caused by belowground C input by plants from root exudates and root death, which is supported by studies showing increased soil microbial biomass after cutting of grassland plants (Guitian and Bardgett 2000; Mawdsley and Bardgett 1997). This effect was smaller after the first and even absent after the second harvest, which we attribute to the high plant growth rate after these harvests (Fig. 3) and thus a strong competition for available P between microorganisms and plants. This is corroborated by the fact that P_{res} decreased after the first and second harvests, whereas it increased after the third (Fig. 2). Although microorganisms can potentially outcompete plants with respect to P uptake (Rousk et al. 2007), their competitiveness is governed by C availability which can be regulated by plants (Marschner et al. 2011). In addition, the unidirectional flow of nutrients from the soil to the roots gives plants a competitive advantage over time (Kuzyakov and Xu 2013).

Seasonal fluctuations in P_{res} (CV 30–46 %) were greater than those in P_{hex} and negatively related to soil moisture (Table 2). Under laboratory conditions, P_{hex} and P_{res} were negatively correlated to soil moisture contents of 0.02–0.40 g g^{-1}, suggesting that the majority of the released P originated from the microbial biomass, even though abiotic contributions due to aggregate disruption were also identified

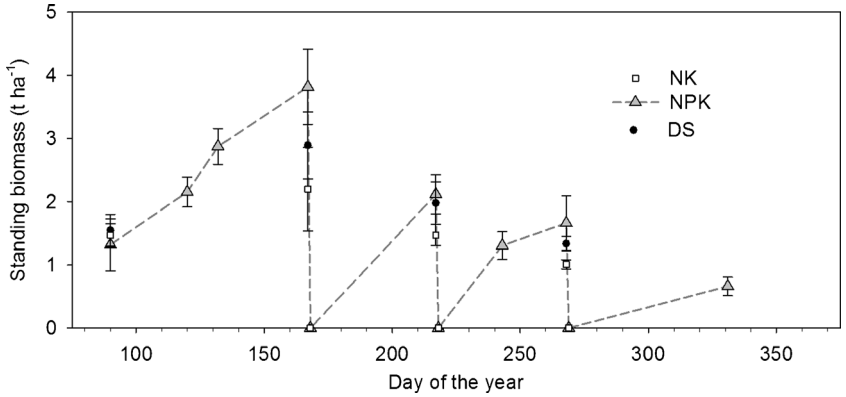

Fig. 3 Aboveground (cut above 4 cm) biomass (t dry matter ha^{-1}) in the three fertilizer treatments NK, NPK and DS in 2009, with more frequent sampling in NPK than in NK and DS. Means ± standard deviations (n =4)

Table 3 Mean seasonal hexanol-labile P (P_{hex}), calculated P_{hex} turnover and fluxes in the top 5 cm of the soil, and stocks of resin-extractable P (P_{res}) and P_{hex} compared to total seasonal plant P uptake as affected by the three fertilization treatments NK, NPK and DS

		NK	NPK	DS	LSD
Mean P_{hex}	(mg kg^{-1})	34.6±4.6	47.8±9.2	70.0±1.7	11.4
∑ of P_{hex} losses	(mg kg^{-1})	34.5±12.2	52.1±9.7	70.4±16.8	21.5
P_{hex} turnover rate	($season^{-1}$)	1.03±0.43	1.11±0.24	1.00±0.23	0.54
P_{hex} turnover time	(days)	293±194	224±47	251±64	212
P_{hex} flux	(kg ha^{-1} $season^{-1}$)	18.1±6.4	27.4±5.1	36.9±8.8	11.3
Mean P_{res} stock	(kg ha^{-1})	0.9±0.1	5.9±0.8	2.6±0.4	1.1
Mean P_{hex} stock	(kg ha^{-1})	18.2±2.4	25.1±4.8	36.7±0.9	6.0
Total plant P uptake	(kg ha^{-1} $year^{-1}$)	6.2±1.1	16.6±1.4	11.7±1.5	2.7

Means ± standard deviations (n=4). Least significant differences (LSD) calculated for α=0.05, with Holm adjusted p values

(Bünemann et al. 2013). In the field, the absence of a correlation between P_{hex} and P_{res} (Table 2) points to non-microbial factors governing P_{res}. Magid and Nielsen (1992) suggested that microscale changes in redox status of ferric minerals may affect the extractability of inorganic P. However, such effects were negligible in well-drained soils (Henderson et al. 2012). More importantly, plant P uptake may be quite sensitive to soil moisture content, which may affect the competition between plants and microorganisms for P_{res}. Indeed, Jupp and Newman (1987) observed cessation of P uptake by *Lolium perenne* at gravimetric soil water contents below 0.20 g g^{-1}, equivalent to soil matric potentials of about −6,000 hPa. In our soil, this corresponds to gravimetric soil water contents of 0.06–0.14 g g^{-1} as determined with a soil water retention curve (Fig. 3, supplementary electronic material). This curve also indicates that a reduction in P uptake due to limited diffusion could be expected below gravimetric water contents of about 0.30 g g^{-1}, equivalent to about −1,000 hPa, since field capacity extends only to pF 2.5 (=−316 hPa). Thus, the transient increases in P_{res} on days 110, 155, 187 and 243 could be explained by a transient decrease in plant P uptake during dry periods, whereas the maxima in P_{res} on day 288 could be attributed to the very low plant P uptake after the third harvest as discussed above.

Table 4 Effect of sampling frequency on P_{hex} turnover rate during the season of 2009

Scenario	P_{hex} turnover rate ($season^{-1}$)		
	NK	NPK	DS
A (n=12)	0.95±0.06	1.03±0.05	0.90±0.08
B (n=9)	0.81±0.34	0.87±0.28	0.85±0.28
C (n=5)	0.41±0.19	0.36±0.07	0.24±0.09

Scenario A (n=12): Turnover rate was calculated based on 12 sampling times, for all 13 possible combinations, i.e. excluding each of the 13 sampling times once. Scenario B (n=9): One sampling per month (days 90, 110, 132, 155, 185, 222, 267, 288 and 331). Scenario C (n=5): One sampling every 2 months (days 90, 132, 185, 267 and 331). Means ± standard deviations of 13 different estimates (A) or of 4 field replicates (B and C)

Turnover rate of P_{hex}

Our hypothesis of faster microbial P turnover in NK was based on our previous tracer-based finding of much faster and greater microbial P immobilization in the absence than in the presence of P fertilization (Bünemann et al. 2012). However, the turnover of P_{hex} calculated from the seasonal dynamics of P_{hex} was similar in all the three fertilization treatments (Table 3), rendering P_{hex} turnover times of 224–293 days, compared to the tracer-based estimates of about 5 and 90 days in NK and NPK, respectively. Thus, it is possible that microbial tracer uptake overestimates P_{hex} turnover, especially under P limitation, when high affinity P transporters enable microorganisms to take up the tracer very efficiently (Wanner 1996). This would lead to rapid microbial P uptake shortly after soil disturbance due to labeling when the specific activity of the soil solution is still very high and would indicate a lack of continuing exchange between microorganisms and the soil solution afterwards. Based on a modeling study, Achat et al. (2010b) postulated that the microbial P pool may consist of two pools with different turnover times and that the one with a very fast turnover of 5–9 days soon became persistent, retaining the immobilized tracer.

The estimation of the P_{hex} turnover rate based on net changes in P_{hex} over time is likewise problematic. As pointed out by Harden and Joergensen (2000), it may be affected by the spatial variability of microbial biomass measurements. If this was not eliminated by our sampling scheme, it would have caused an overestimation of the turnover rate. The P_{hex} turnover rate may also largely depend on the number and selection of sampling times. To check for the influence of each specific sampling date, we calculated the P_{hex} turnover

rate again based on 12 sampling dates, i.e. leaving out each of the 13 sampling dates once (scenario A, Table 4). This resulted in slightly reduced turnover rates (0.9–1.0 season^{-1}) which were still similar between the treatments. Leaving out the final sampling date with a consistent decrease in P_{hex} in all treatments decreased the turnover rates most. Similarly, an increase in sampling dates would have increased the P_{hex} turnover rate, especially if we missed major maxima or minima with our current sampling design. Calculating the P_{hex} turnover based on gains rather than losses in P_{hex} between two sampling times resulted in similar estimates of P_{hex} turnover to the values in Table 3 (data not shown). However, reducing the sampling times to one sampling each month (scenario B) or every 2 months (scenario C) further reduced the P_{hex} turnover rate, especially in scenario C (Table 4). This agrees with the theoretical considerations of Harden and Joergensen (2000) that microbial turnover will increase with the number of sampling times.

We conclude that microbial P turnover rates derived from seasonal dynamics can hardly be compared between studies which use different numbers of sampling times. Unfortunately, a direct comparison of the approach by McGill et al. (1986) to radiotracer-based approaches is hampered by problems of applying radioisotopes in the field and by the steady-state assumption in isotopic dilution experiments. An alternative method could be the analysis of the isotopic composition of oxygen in phosphate in different soil P pools in combination with modeling (Tamburini et al. 2012). In very young soils in a glacier forefield, this approach showed a fast microbial P turnover time of 20 days to be most likely. A comparison to the McGill approach across different ecosystems would be valuable, especially since the oxygen isotope approach potentially integrates the seasonal dynamics occurring in the field.

Implications for P availability and plant P uptake

After 17 years of different P fertilization regimes, significant differences in available P had developed in the investigated topsoil layer, with average P_{res} of 1.8, 4.9 and 11.2 mg P kg^{-1} in NK, DS and NPK, respectively. Analysis of P_{res} in deeper soil layers (5–10 cm and 10–20 cm) did not reveal significant differences between treatments (data not shown). Therefore, we limit our discussion of P availability to this topsoil layer. Importantly, P deficiency in NK and DS was indicated by the fact that dry matter yields in NK and DS reached only 63 and 83 % of the yield in NPK (Fig. 3) and that seasonal P uptake in NK and DS was 37 and 70 % of that in NPK (Table 3). Plant N limitation in DS is unlikely based on the finding that plant N concentrations in DS were similar to those in NPK, while plant P concentrations were reduced in DS (Liebisch et al. 2013).

At the same time, the mean P_{hex} stock was greatest in DS and smallest in NK, and due to the similar P_{hex} turnover rate in all the treatments, the P_{hex} flux ranged also in the order DS> NPK>NK (Table 3). P_{hex} stock and flux in the topsoil were 1.5–3 times greater than seasonal plant P uptake. However, we cannot deduce an effect of this P_{hex} flux on plant P uptake, which ranged in the same treatment order as P_{res} and not as P_{hex} flux. Likewise, the greater potential organic P mineralization indicated by greater acid phosphomonoesterase activity in NK than NPK (Fig. 2) is in accordance with the differences observed in gross and net P mineralization rates (Bünemann et al. 2012), but did not seem to improve plant P availability in NK. The lower acid phosphomonoesterase activity in NPK may also be attributed to the inhibition of phosphomonoesterase activity by inorganic P (Nannipieri et al. 1978) or to changes in the bacterial community composition, with potential implications for the expression of functional genes such as alkaline phosphatase genes (Tan et al. 2013). The lowest activity in DS may be caused by differences in soil pH (Table 1), with the relative importance of acid vs. alkaline phosphomonoesterase decreasing as pH increases. Our observations are in contrast to Simpson et al. (2012) who compared two soils with similar inorganic P status and observed greater plant P uptake from the soil which had higher concentrations of organic P, suggesting an important role for soil biological processes in P nutrition of grasslands.

Finally, the relatively large fluctuations in P_{res} illustrate the difficulty to recommend suitable sampling periods to estimate P availability as a basis for fertilization recommendations. Our data suggest that the maxima of P_{res} can be expected when sampling is done during or shortly after dry periods, while minima are likely to occur during periods with high plant P uptake rates. At least in Central Europe, the best sampling time may be during winter when soils are usually moist, and plant uptake is negligible.

Conclusions

Different P fertilization strategies in an extensively managed permanent grassland in Switzerland affected plant P uptake and P_{res}, both ranging in the order NPK>DS>NK. In contrast, the turnover rate of P_{hex} as deduced from the temporal fluctuations in P_{hex} was similar in all treatments (approximately once per growing season). The seasonal flux of P through the microbial biomass was therefore similar to the stock of P_{hex}, which ranged in the order DS>NPK>NK and was 1.5–3 times greater than plant P uptake, even though only the top 5 cm of the soil was analysed. While the estimation of P_{hex} turnover rates based on seasonal dynamics has severe drawbacks, such sampling schemes are still valuable for a better understanding of soil P dynamics. In our study, fluctuations in P_{res} and P_{hex} pointed to strong interactions between plants and microorganisms, driven by soil moisture and presumably also by alterations in belowground C inputs following harvests. To

better understand these interactions between plants, microorganisms and environmental factors, soil P and C dynamics as well as plant growth should be studied at greater temporal and spatial resolution.

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