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PLANT AND ENVIRONEMENT INTERACTIONS

Phytoextraction of Soil Phosphorus by Potassium-Fertilized Grass-Clover Swards

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Abstract

In the development of the Dutch National Ecological Network, many hectares of arable land are converted to nature areas to protect plant and animal species. This encompasses development of species-rich grasslands. On former agricultural land on sandy soils, this development is often hampered by relatively high phosphorus (P) levels, which also cause eutrophication. Standard practices to decrease the amount of P are either topsoil removal or long-term mowing of low-yielding established grassland. Both methods have disadvantages, and there is a need for additional techniques. As an alternative, phytoextraction ("mining") of soil P has been proposed. We tested a new technique of mining without mineral N fertilizer by cropping an intensively mown grass-clover with potassium (K) fertilization that could potentially be used as cattle feed. A long-term field experiment was conducted, comparing soil P removal by grass-clover swards with and without supplementary K fertilization on a sandy soil. During the experiment, which ran from 2002 to 2009, soil P levels and nutrient contents of grass-clover were measured, and P and K balances were calculated. Our results show that grass-clover with K fertilization removed excess soil P (also at lower P levels) at a relatively high rate (34 kg P ha^{-1} yr^{-1}, significantly higher than without K fertilization; $P < 0.05$) and produced reasonable yields of grass-clover. Our P balance suggested reduced leaching from the topsoil during this experiment. For nature restoration in agricultural areas, this tool opens many possibilities.

Core Ideas

- Intensively harvested grass-clover is an effective tool to reduce excess P from topsoil.
- On sandy soils a potassium source is necessary for this technique to be successful.
- Soil P phytoextraction ("mining") can reduce P enough for species-rich grassland development.
- Soil balances suggest reduced leaching of P, declining the P load to surface water.

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On a global scale, awareness is growing that the amount of phosphorus (P) available for fertilizer production and crop growth is limited and that a potential P crisis is looming (Abelson, 1999; Herring and Fantel, 1993). At the same time, some western European countries, including The Netherlands, are having problems with excessive P levels in agricultural soils. In The Netherlands, an extensive network of green corridors, called the National Ecological Network, is being implemented (Bakker et al., 2015). The idea originated in the 1990s and aims to protect species of animals and plants. The National Ecological Network encompasses various types of nature areas. In this context, many hectares of arable land have been transferred to nature reservations, and many more are planned (originally the plan was to implement 728,500 ha in 2018, but the ambition has decreased somewhat in terms of area and planning). In most of these former agricultural soils, long-term applications of fertilizers and animal manure in the past, which were stopped after land acquisition by nature organizations, have led to an imbalance in the levels of soil nutrients. Although these soils often have high levels of relatively immobile soil P, the more mobile nitrogen (N) and potassium (K) have often become suboptimal according to agricultural standards because of the lack of manure for some years. The cause and extent of this problem is not limited to The Netherlands; all countries of the European Union have a positive P balance because P inputs via fertilizers and animal feed far exceeded P outputs in agricultural products (Isermann, 1990; Bennett et al., 2001). Several more recent studies show the excessive use of P fertilizer in western Europe over the last decades (Schoumans et al., 2015; Ott and Rechberger, 2012; Csathó and Radimszky, 2009). Schoumans et al. (2015) show that the European Union members were the largest consumer of P fertilizer in the period 1961 to 2012, although consumption has declined since the 1970s and especially since the recent price peak (2008–2009). These authors pointed out P accumulation in agricultural soils, environmental threats, and the need for P recycling. Schoumans and Chardon (2015) showed that over 60% of all arable noncalcareous sandy soils in The Netherlands had a P saturation rate higher than 25%, which they calculated to be the "critical" soil saturation level above which P would eventually leach into the groundwater.

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Abbreviations: DM, dry matter; P-AL, ammonium lactate–extractable phosphorus; P-total, total amount of soil P after destruction with sulfuric acid; Pw, water-extractable phosphorus; SOM, soil organic matter.

In addition to problems with leaching and eutrophication of surface water, high nutrient levels are a threat to certain types of species-rich plant communities (Berendse et al., 1992). For example, high soil P levels are associated with low plant species diversity in grasslands, whether this effect is direct or indirect (Ceulemans et al., 2014; Critchley et al., 2002; Janssens et al., 1998). However, the relations presented in these studies show a wide range of plant diversity at low P levels, pointing out that other factors (e.g., hydrology and historic and current management) are also of influence. In a broad study across temperate Europe, Wassen et al. (2005) showed that many more endangered plant species persist under P-limited conditions than under N-limited conditions and hence deduced that P enrichment is more likely to be the cause of plant species diversity loss than N enrichment. Moreover, Fujita et al. (2014) showed that many endangered plant species are adapted to P limitation and hence are associated with P-limited conditions. The low investment of such species in sexual reproduction further threatens their survival in P-rich soils. In The Netherlands, extensively managed, somewhat wet grasslands with high soil P levels often develop a vegetation dominated by one single species (e.g., *Juncus effuses* L.) depending on hydrology and management (Smolders et al., 2008; Geurts et al., 2011). *Juncus effusus* is a common, grass-like perennial that can stay dominant for years and is typical for relative species-poor grasslands with little agronomical or ecological value.

One of the methods to decrease soil P levels for nature restoration is topsoil removal (Smolders et al., 2008), but this practice is often expensive and conflicts with the objectives of soil conservation and conservation of historical heritage. A thorough study concerning local hydrology, depth up to which soil P levels are high, depth of soil removal, and (re)colonization potential (e.g., the presence of target species in the surrounding area) is needed to see if and how such a measure can be applied successfully. Topsoil removal leads to loss of organic matter (SOM), soil biota, and soil structure.

Other methods to remove readily available soil P (e.g., fixing P with lime or bentonite clay [Geurts et al., 2011]) are not always effective or practical because they could decrease P mobilization to water but without decreasing Olsen-P concentrations or growth of *J. effusus*. Nature conservation organizations often turn to sowing perennial grass swards and removing nutrients through repeated mowing with little or no fertilizer or manure application. Typically, dry matter (DM) production of these grasslands will strongly decrease after some years, primarily due to N limitation. This decreased production subsequently inhibits further P removal. On sandy soils, this practice may also enhance noxious weeds (e.g., ragwort [*Senecio jacobaea* L.]), and it may limit the amount of soil organisms that are a food source for birds and mammals (e.g., blacktailed godwit, curlew, and badger).

Hence, there is an urgent need for a better strategy to remove excess P from former agricultural land in a way that ensures successful development of ecosystem target types, such as nutrient-poor, species-rich grasslands. Various pot experiments have shown that phytoextraction of soil P (also called "mining" of P) leads to a rapid reduction in the readily available soil P fractions at zero P application (Yli-Halla et al., 2002; Koopmans et al., 2004a, 2004b). Field studies are relatively rare. As described by Marrs (1985), a cereal crop (rye) was successfully tested as a means to reduce soil nutrient levels, showing that more P was removed when the crop received N fertilizer. However, cereal grains such as rye are annual crops and require replanting for phytoremediation to be effective. Field experiments in The Netherlands (Van der Salm et al., 2009) studied phytoextraction with a productive grass sward (90% perennial ryegrass [*Lolium perenne*] L. or 35% *L. perenne*, 20% *Poa trivialis* L., and 15% *Agrostis stolonifera* L.) to reduce leaching of P to surface water. They showed that a perennial grassland in combination with relatively high rates of N fertilization offers a possibility to lower the P concentration in the soil solution and the readily available soil P in the topsoil.

From this starting point, we wanted to design and test a method for lowering soil P to develop species-rich grassland in a nature reserve. Within nature reservations, risks of eutrophication with N and P should be kept as low as possible. Therefore, the policies of Dutch nature organizations include strong restrictions in the use of N fertilizers and manure in many areas. However, without a N source, we would foresee relative low grassland productivity on Dutch sandy soils. Therefore, we designed a method to test phytoextraction of P in the absence of mineral N application but with grass-clover swards to provide N to the system.

However, experiences in practice in various nature areas using grass-clover swards (predominantly *L. perenne* with white clover [*Trifolium repens* L.] and some also with *Trifolium pretense* L.) resulted in losing the clover from the sward and very low DM yields after some years. We argued that legumes are weak competitors for K (Blaser and Brady, 1950; Mengel and Steffens, 1985) and hypothesized that (i) K limitation could be the cause of clover decline in grass-clover swards in nature areas and that (ii) with K fertilization, grass-clover swards could be an effective method to reduce soil P levels in sandy soils to levels well below agricultural advice values and even to levels low enough to accommodate species-rich plant communities. We designed a field experiment with a duration of 7 yr to test these hypotheses, with the aim to develop a new and cost-effective way to remove excess P from former agricultural soils by phytoextraction of P in the absence of mineral N fertilizer but with a grass-clover sward as an N source. Measurements of soil P levels at the end of the experiment are compared with some reference measurements in nearby grasslands.

Materials and Methods

Experimental Design

In a field experiment, two treatments were compared: (i) no fertilizer application and (ii) K fertilizer application at a rate of 398 kg K ha^{-1} yr^{-1}. Our experiment was set up on a former arable field that is currently part of a nature conservation area in the south of The Netherlands (Hengstven, part of the Loonse and Drunense Duinen national park, in the province of Noord Brabant [51°38.904′ N, 05°11.918′ E]). During the time of the experiment (2003–2009), the area had an average annual temperature of 10.8°C and on average 786 mm of annual rainfall (Royal Meteorological Institute, 2012). In this area, nature conservation organizations aim to develop species-rich grasslands, wet heather vegetation, and fens. For these ecosystem types, soil P levels were much too high. The field with the experiment had a noncalcareous sandy soil (Spodosol; Podzol, cover sands of pleistocene origin; 5.8% SOM in the topsoil, pH-K-Cl of 4.3) with P-total (i.e., the total amount of soil P after destruction with sulfuric acid) levels of 362 mg P kg^{-1} of dry soil, an ammonium lactate–extractable P (P-AL) value of 131 mg P kg^{-1} of dry soil, and a P saturation level of 0.36 in the 0- to 10-cm layer. The P-AL level at that time could be classified as “optimal” according to the standard for arable land used by the Belgian Pedological Service (De Smet et al., 1996). Before our experiment, the field had been cropped with maize for many years. Fertilization was done with cattle slurry at a medium level (not too much slurry was applied because the field was located relatively far from the farmer and transport costs were high). After the maize, in 2001, an unfertilized barley crop (*Hordeum vulgare* L.) was grown.

The experimental plots were sown in 2002, and treatments and measurements continued until 2009. The experimental design was a randomized block design in four blocks with four replicate plots (4 × 10 m) per treatment. All plots were sown with a mixture of 30 kg ha^{-1} *L. perenne* and 4 kg ha^{-1} *T. repens*. Each year, K fertilizer (based on potassium sulfate) was applied in four portions: 149 kg K ha^{-1} in early spring 6 to 8 wk before the first harvest and three applications of 83 kg K ha^{-1} each, the week after the second, third, and fourth harvest. In some years, a fifth harvest was conducted, but no additional fertilizer was applied. The fertilizer was spread out over the swards and was not incorporated into the soil.

Measurements

At each harvest date, all plots were mown to a cutting height of 5 cm with a two-wheel tractor (Eurosystems P55) equipped with a cutter bar. For each plot, freshly mown material from the plot center (∼4 m^2) was weighed immediately on site to determine fresh yield. A representative fresh sample of about 700 g was dried at 70°C for 24 h. A subsample of these dried samples was analyzed for P and K contents (total P and total K, destruction with nitric acid, measurement with inductively coupled plasma–atomic emission spectroscopy) by a commercial laboratory (BLGG AgroXpertus). Another subsample was dried at 105°C for 1 h additionally to determine DM content and to calculate total DM yield. In each plot, a second fresh sample of about 300 g was hand sorted into three categories: white clover, grasses, and dicots other than clover. The sorted material was dried separately to allow calculation of relative white clover content per harvest (weight percent of DM yield) and total white clover DM yield per harvest.

Soil samples from the 0- to 10-cm soil layer were taken each year in winter except for the winter of 2003–2004. Soil sampling at the start of the experiment (2002–2003) consisted of taking one composite soil sample of all eight plots measured in later years and in winter 2004–2005 of one composite sample per treatment (combining soil from four repetitions). From winter 2005–2006 onward, all replicate plots were sampled and analyzed individually. Before chemical analysis, soil samples were oven dried at 40°C. Several P fractions were measured in soil samples: P-AL was measured according to Egnér et al. (1960), water-extractable P (Pw) was measured according to Sissingh (1971), total P was measured with Fleishmann acid (Houba et al., 1997), and the P saturation at the start of the experiment was measured according to Schwertmann (1964). Also measured was the K-HCl, which was determined after soil extraction with HCl (0.01 mol L^{-1}), and the pH-KCl, which was measured at the beginning of the experiment in a 1:10 (v/v) suspension in KCl_2 (0.1 mol L^{-1}). Soil organic matter was determined by loss-on-ignition at 555°C (Ball, 1964).

To compare soil data from the experimental plots with “reference” grasslands, soil samples were taken in six perennial grasslands in nature conservation areas with varying plant species diversity. These grassland were located on noncalcareous sandy soils in the same region. These soil samples were taken in 5 × 5 m plots in the winter of 2007 and analyzed for P-AL concentration on the same date as the soil sampling in the experimental plots and with the same procedure. In June 2007, vegetation in the reference grasslands was analyzed with the Braun–Blanquet method adapted by Barkman et al. (1964) in the same 5 m × 5 m plots as used for the soil sampling. At the same time, the vegetation in the experimental plots was analyzed using the same method.

Calculations and Statistics

For the calculation of the K and P balances, the top 10 cm of the soil was considered. Bulk density was estimated from measured data of SOM during the experiment using an empirical formula for Dutch sandy soils (Bemestingsadvies, 2012):

$$\text{bulk density (kg dm}^{-3}) = 1/(0.02525 \times \text{SOM} + 0.6541)$$

Statistical analysis was performed using GenStat statistical software (version 13.3, VSN International Ltd.). Repeated measures ANOVA was used for comparing annual data across years and treatments. Significant differences were determined based on least significant differences ($p < 0.05$).

Results

Grass-Clover Production and P Removal

In 2002 (year of sowing) and 2003, white clover abundance was comparably high in both treatments (i.e., with and without K fertilizer) (Fig. 1). In the following years, until 2008, clover

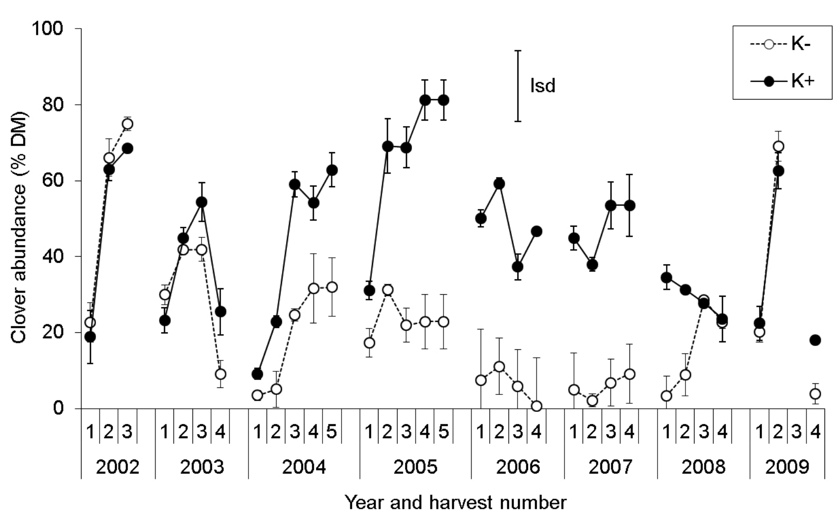

Fig. 1. Average clover abundance (% of total dry matter [DM] yield; $p = 0.002$) in K-fertilized plots and unfertilized plots from 2002 to 2009. Error bars indicate SEM. The least significant difference (lsd) ($p < 0.05$) is indicated by the error bar at the top.

abundance was significantly ($p = 0.002$) lower in the treatment without K fertilization. In 2008 and 2009, the difference between the fertilized and unfertilized treatment was not significant.

Similar to clover content, total DM yield (clover plus grass) was similar between treatments in the first 2 yr of the experiment but was significantly lower in the unfertilized plots from 2004 onward until the end of the experiment in 2009 (Fig. 2A). In 2006 and 2007, yields differed by more than a factor of two.

The amount of P removed by grass-clover was significantly higher in the K-fertilized treatment than in the unfertilized treatment during most years of the experiment (2005–2008) (Fig. 2B). Compared with differences in DM yield, differences in P removal were somewhat smaller between treatments because P content (g kg^{-1} DM) in the treatment without K fertilization increased with decreasing biomass ($p < 0.001$; $R^2 = 0.18$) (Fig. 3).

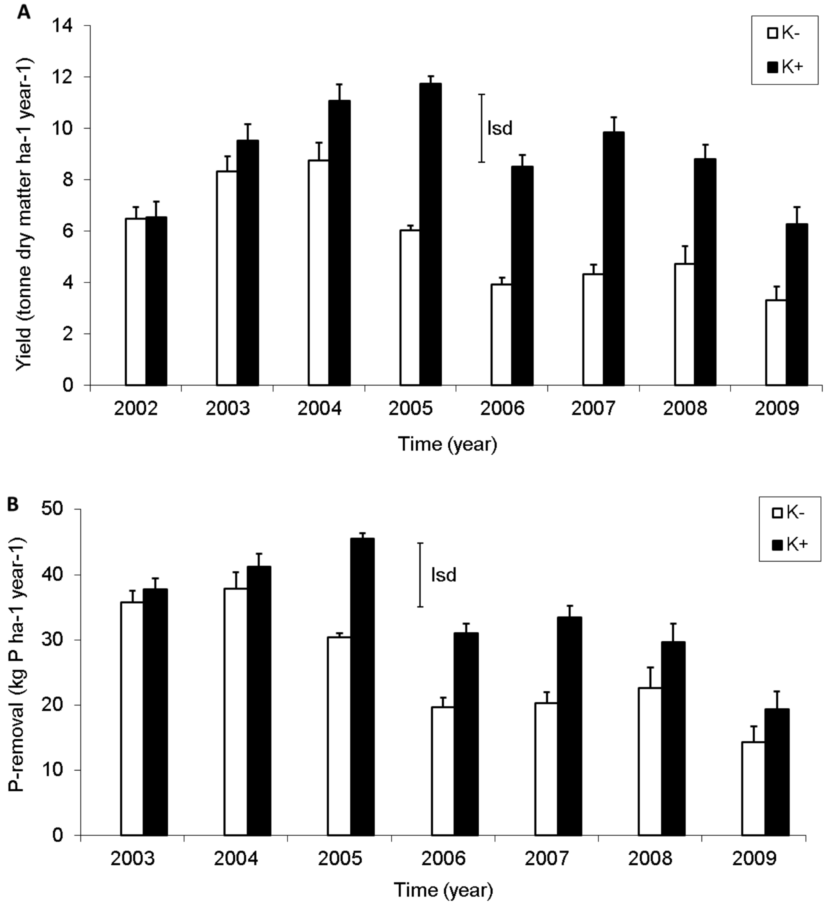

Fig. 2. Average annual dry matter yield ($p = 0.004$) (A) and P removed by mowing the grass-clover crop ($p = 0.05$) (B) in K-fertilized plots and unfertilized plots from 2002 to 2009. In 2009 one harvest was not analyzed, resulting in a lower total annual yield and P removal measured. Error bars indicate SEM. The error bar at the top indicates the least significant difference (lsd) ($p < 0.05$).

Soil Nutrients and Nutrient Balances

Chemical analyses of soil samples from the experimental plots showed significant decreases in P-total, P-AL, and Pw in both treatments over the years (Fig. 4A–C). For example, P-AL, a commonly used indicator of plant-available P in grassland in The Netherlands, decreased from 127 mg P kg^{-1} dry soil in 2003 to 35 mg P kg^{-1} in 2009 in the treatment with K fertilization. Soil P levels (P-total, P-AL, Pw) were generally lower in K-fertilized plots than in unfertilized plots, but this treatment effect was not significant ($p = 0.085$, $p = 0.107$, and $p = 0.135$). In contrast, soil K (K-HCl) differed significantly between treatments but not between years: it was always higher in K-fertilized plots ($p = 0.005$) but did not vary significantly over the years ($p = 0.365$) (Fig. 4D). Finally, SOM showed an increasing trend over the years of the experiment ($p = 0.058$). This trend appeared to be stronger for the K-fertilized treatment (Fig. 4E), but there was no significant treatment effect. However, linear regression analysis showed a significant SOM increase in the fertilized treatment ($p = 0.02$; slope, 0.27 yr^{-1}; SE, 0.06), whereas no such effect was found for the unfertilized treatment.

Based on these soil data and the measured nutrient content of harvested plant material, we calculated K and P balances for both treatments. The soil K balance (Table 1) shows that the amount of K removed by grass-clover harvesting exceeded the amount of K added through fertilizer. The soil P balance (Table 2) shows that, over the 7 yr of the experiment, K-fertilized grass-clover had removed more P from the topsoil than unfertilized grass-clover ($p = 0.003$): 238 kg P ha^{-1} (averaging 34 kg P ha^{-1} yr^{-1}) compared with 181 kg P ha^{-1} (26 kg P ha^{-1} yr^{-1}). In the K-fertilized treatment, the difference between soil P content at the start of the experiment and total P removed by grass-clover harvesting was practically equal to soil P content at the end of the experiment: the balance difference was only 8 kg P ha^{-1} over 7 yr. However, in the unfertilized treatment this difference was much larger. Here, an additional amount of 59 kg P ha^{-1} (8 kg P ha^{-1} yr^{-1}) was missing in measurements of total P in the topsoil after 7 yr.

Botanical Composition in Relation to Soil P

The relation between soil P and plant species number in six reference grasslands in nature reserves shows that species number decreased with increasing soil P ($p = 0.04$; $R^2 = 0.69$) (Fig. 5). Because the ecological value of perennial grasslands not

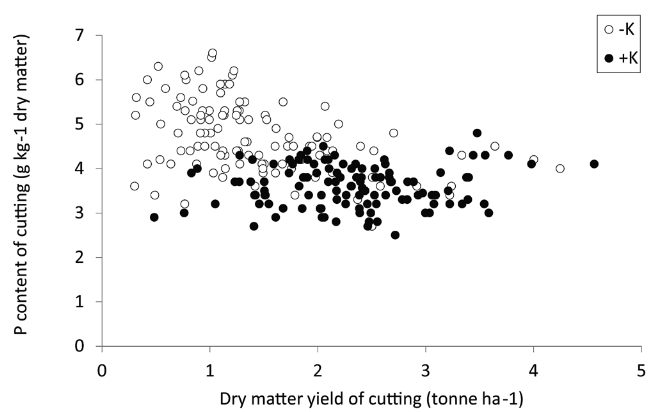

Fig. 3. Phosphorus content of harvested above-ground plant material plotted against dry matter yield of each replicate plot and harvest date shown for K-fertilized plots and unfertilized plots. For the latter, there was a significant relation ($p < 0.001$; $R^2 = 0.18$).

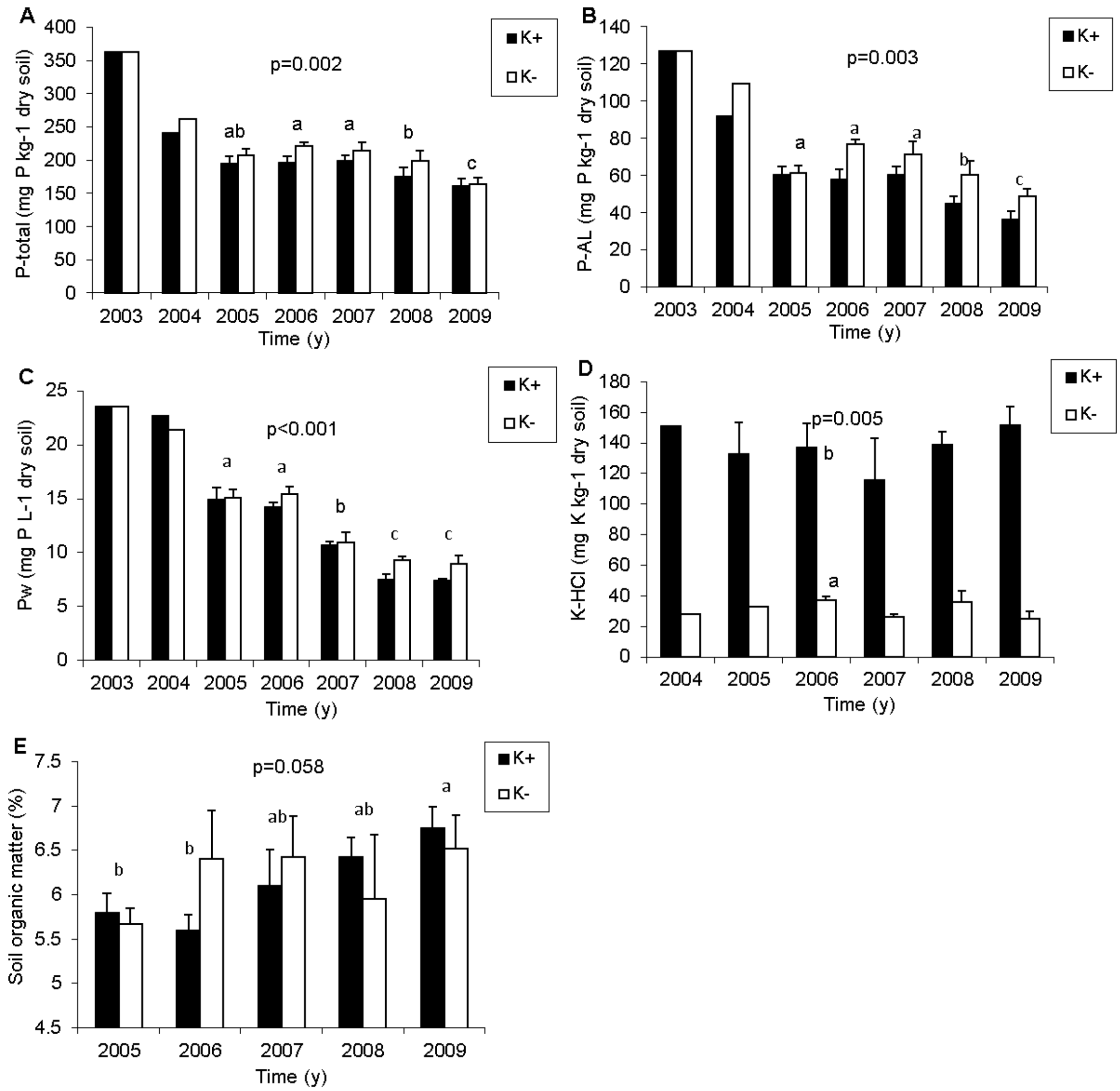

Fig. 4. Average total amount of soil P after destruction with sulfuric acid (P-total) (A), ammonium lactate–extractable P (P-AL) (B), water-extractable P (Pw) (C), K-HCl (D), and soil organic matter (E) in the 0- to 10-cm soil layer of K-fertilized plots and unfertilized plots based on annual sampling during 2003–2009. Repeated measures ANOVA revealed significant time effects for P-total, P-AL, and Pw (see *p* values in A, B, and C) and a significant treatment effect for soil K (see *p* value in D). Different letters indicate significant differences based on repeated measures analysis. Error bars indicate SEM. In the first 2 yr of soil sampling (2003, 2004), P-total, P-AL, and Pw were measured only in composite soil samples, not in individual plots. These data were not included in the statistical analysis and hence are shown in this graph without error bars.

only depends on the number of species but also on the identity of the species present and their (relative) abundance, we also described the vegetation of the reference grasslands (Table 3). As Table 3 shows, our reference sites included two typical species-rich grasslands with several rare species present in high abundance, such as the hemiparasites *Rhinanthus angustifolius* C.C. Gmelin and *Pedicularis palustris* L. and the orchid *Dactylorhiza majalis* (Rchb.) P.F.Hunt & Summerh., which are on the Dutch red list of endangered species (Grasslands 1 and 2 in Table 3). Two other grasslands (Grasslands 3 and 4 in Table 3) were also relatively species rich, but none of the endangered species mentioned above was present. Instead, high numbers of more common species, such as *Juncus acutiflorus* Ehrh. ex Hoffm and *Lychnis flos-cuculi* L., which are indicative of relatively wet and somewhat less productive grasslands, were found. The fifth grassland was a "typical P-rich grassland" with only three species in high numbers (*J. effusus* being the dominant species). Finally, the sixth grassland was even richer in soil P than the fifth but still had a relatively diverse vegetation and even a few *P. palustris*. Compared with the soil P-AL levels in these reference grasslands, P-AL levels in our experimental plots were intermediate at the

Table 1. Soil K balance over 2004 to 2009 for each treatment.

Treatment	K–	K+
	kg K ha^{-1}	
K input from fertilizer	0	1991
K removal by grass-clover†	535 (62)‡	2321 (107)
Soil K-HCl§		
2004	41	228
2009	56 (8.4)	205 (22)

† Potassium removal by grass-clover was calculated based on total dry matter yield and measured K content of the harvested above-ground plant material for each harvest during 5 yr.

‡ Numbers in parentheses indicate SEM.

§ Soil K levels were measured in the topsoil (0–10 cm depth).

Table 2. Soil P balance over 2003 to 2009 for each treatment.

Treatment	K–	K+
	kg P ha^{-1}	
Measured soil P-total 2003	444	444
P removal by grass-clover†	181 (11)‡	238 (11)
Expected soil P-total 2009§	264	206
Measured soil P-total 2009	205 (14)	198 (12)
Difference¶	−59	−8

† Phosphorus removal by grass-clover was calculated based on total dry matter yield and measured P content of the harvested above-ground plant material for each harvest during 6 yr.

‡ Numbers in parentheses indicate SEM.

§ Soil P levels were measured in the topsoil (0–10 cm depth). P-total, total amount of soil P after destruction with sulfuric acid.

¶ Comparison of measured P-total in 2009 and expected P-total in 2009 based on the difference between measured P-total in 2003 and total P removed by grass-clover over 2003 to 2009.

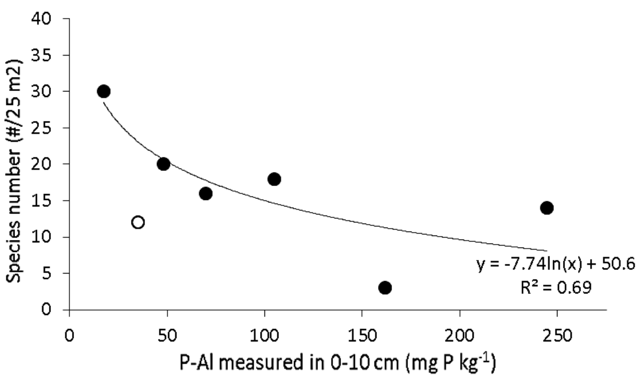

Fig. 5. Species number (Braun Blanquet method on 25-m^2 plots, June 2007) in six natural "reference" grasslands on sandy soils, plotted against ammonium lactate–extractable P (P-AL) in the topsoil (0–10 cm depth) (solid dots). The open dot ('exp') indicates the species number and P-AL level in the K fertilized treatment by the end of the experiment in 2009 (n = 12 species; SE, 0.35). Numbers refer to the grasslands described in Table 3. ***Linear regression between species number and log-transformed P-AL content was significant (p* = 0.04) **(slope, intercept, and** R^2 **are shown in the graph).**

start of the experiment in 2002 (when P-AL was 127 mg P kg^{-1}; i.e., in between values measured in reference Grasslands 4 and 5). By the end of the experiment in 2009, P-AL in the K-fertilized treatment had decreased to 35 mg P kg^{-1}, which is between the P-AL values measured in Grasslands 1 and 2. However, species number in this treatment by 2009 was still much lower than in these reference grasslands and was also lower than predicted by the statistical relation between species number and P-AL content (12 species per plot instead of 23) (Fig. 5).

Discussion

Our results indicate that our first hypothesis was correct: in plots without K fertilization, relative clover abundance and total productivity of grass-clover were lower than in K-fertilized plots, in particular after the first 2 or 3 yr after establishment. This suggests that K uptake indeed becomes limiting for clover (and hence, because clover is the N source, also for total DM production and P removal) after repeated harvesting of grass-clover swards on P-rich sandy soils. Our results also support the first part of our second hypothesis (i.e., that grass-clover combined with K fertilization can be an effective method to reduce soil P levels in sandy soils); this treatment removed 34 kg P ha^{-1} yr^{-1} on average. In comparison, the amount of P removed with a productive grass sward with mineral N fertilization varied from 28 to 50 kg P ha^{-1} yr^{-1} (Van der Salm et al., 2009) and was of comparable size. Whether this rate of removal is high enough to arrive at a low P concentration in a few years remains a field-specific issue. In our field it was surely so, but other soils can have higher P loading, higher P buffering capacity, or both. In such soils, long periods of P removal with grass-clover would be needed to substantially lower the P concentration.

Our results also provide evidence for the second part of this hypothesis: this method could indeed remove enough P to arrive at a plant-available P concentration low enough for species-rich grasslands. By the end of the experiment, the average soil P level in the K-fertilized treatment was comparable to soil P levels in two nutrient-poor, species-rich grasslands in the region (Fig. 5). However, despite the decreased soil P level, plant species number in the experimental plots was still considerably lower than in the reference grasslands. This is not surprising. As shown by others (Janssens et al., 1998; Critchley et al., 2002), the relation between soil P and plant diversity is multifactorial: a low soil P level does not guarantee a species-rich grassland because other factors may also play a role. We did not expect high plant species numbers in our experimental plots because of the presence of highly productive grass and clover species (competition) and the absence of a seed source (e.g., a local soil seed bank or a nearby species-rich grassland).

Table 3. Description of natural "reference" grasslands shown in Fig. 5.

Grassland number†	P-AL	Description of grassland
	mg P kg^{-1}	
1	17	Typical species-rich wet grassland with rare species such as *Rhinanthus angustifolius* and *Dactylorhiza majalis*
2	48	Typical species-rich wet grassland with rare species such as *Pedicularis palustris* and *Caltha palustris* in high abundance
3	70	Perennial grassland with slightly more common species such as *Juncus acutiflorus*, *Cardamine pratensis*, and *Lychnis flos-cuculi*
4	105	Perennial grassland with more common species such as *Holcus lanatus* and *Poa trivialis* but also some *Juncus acutiflorus*
5	162	Typical species-poor perennial wet grassland dominated by *Juncus effusus* but also including some *Juncus conglomeratis* and *Cirsium arvense*
6	245	Species-poor wet grassland dominated by *Juncus effusus* but also including *Antoxantum odoratum*, *Cardamine pratensis*, and even a few *Pedicularis palustris*

† Grasslands are listed in the order of increasing soil ammonium lactate–extractable phosphorus (P-AL) level.

However, one can wonder whether K fertilization is necessary in a practical strategy for effective P removal on sandy soils. Our data suggest it is not: intensive harvesting of unfertilized grass-clover swards also removed considerable amounts of P (26 kg P ha^{-1} yr^{-1} on average) compared with the treatment with K fertilization. Such a treatment is undesirable from a practical point of view because intensive mowing of relatively unproductive swards is not cost effective. In our experiment, the unfertilized swards removed 25% less P and produced 46% less yield (2003–2009). Moreover, harvesting the fertilized swards is more worthwhile to farmers not only because of the higher productivity but also because of the greater relative abundance of clover, resulting in a higher crude protein content (data not shown, but see Gierus et al. [2012] and Soegaard [2009]) and hence higher nutritional value of the harvested grass-clover. This important advantage allows nature conservation organizations to outsource the management and the harvesting to local farmers, who can use the harvested grass-clover to feed their cattle (for the current Dutch situation with recent financial cutbacks, this is very relevant and has been done to the field surrounding our experimental plots).

Necessity of K fertilization on other soil types than the sandy soil of our experiment may be different. Leaching tends to differ between soil types and regions, and soils may differ in availability and buffering capacity of K. The same holds, for example, for a N source (e.g., it may not be necessary on relatively dry peat soils).

We had multiple reasons to choose grass-clover as a crop for reducing excess soil P. First, grass is known for its large P uptake. Sival and Chardon (2004) showed in a review that intensively mown, N-fertilized grassland had higher P uptake than all other crops included in the comparison, such as potatoes, cabbage, and maize. In line with these figures, Marrs (1985) showed that a cereal crop removed less P (even if all crop residues were included in the measurement) than grass (only 10 kg P ha^{-1}). Second, because grass is a perennial crop, the soil does not need to be tilled every year (meaning less disturbance to soil structure and soil seed bank), and management costs are relatively low. Third, grassland is a good choice because in many cases the ecological objective (or ecosystem type) of agricultural land conversion is to create natural grasslands. Adding white clover is an elegant way to provide the necessary N source. However, a point of concern is that white clover persistence is limited: many cultivars persist only 4 to 5 yr, although some others may persist up to 8 yr (Piano and Annicchiarico, 1995; Woodfield and Caradus, 1996). In our study we used the cultivar "Alice" particularly because of its known persistence; nonetheless, we did observe a significant decline in clover abundance 7 yr after sowing (2008) even in the K-fertilized treatment. It has long been known that white clover has a trade-off between flowering and persistence (e.g., Gibson, 1957). This was also observed in our experiment: 1 yr after the decline, in 2009, clover abundance had recovered in both treatments, probably as a result of high seed production in the K treatment in the previous year. Although we did not measure clover abundance, DM production, and P removal after 2009, we suggest that grass-clover swards used for the purpose of P removal from former agricultural soils would require some maintenance (resowing) every 4 to 8 yr depending on the clover cultivar and weather conditions.

Apart from the need to reduce soil P to allow development of nutrient-poor ecosystems, another good reason to remove excessive soil P is to reduce the long-term risk of P leaching into surface and groundwater (Behrendt and Boekhold, 1993; Koopmans et al., 2004a, 2004b). For both our experimental treatments, we calculated soil P balances for the topsoil over 7 yr. Strikingly, the P balance for the K-fertilized treatment seems to show almost no P loss (i.e., the same amount of P removed by the plant material as was the size of the decrease of total P in the top 10 cm), whereas a substantial amount of soil P (59 kg P ha^{-1}) was lost from the total P pool in the topsoil in the unfertilized treatment. Indeed, the average amount of soil P lost from the topsoil in the unfertilized treatment was 8 kg P ha^{-1} yr^{-1}, which is about equal to the difference between treatments in the amount of P removed through harvesting (9 kg P ha^{-1} yr^{-1}). Within our simple P balance, no uptake of P from deeper layers is taken into account. Although most of the rooting and P uptake will take place in the top 10 cm of the soil considered, grass-clover can take up P also from deeper layers (Goodman and Collison, 1981). This means that our closed P balance is partly due to uptake of P from deeper layers, compensating for the leaching of P from the topsoil. We have three suggestions to explain the missing P in the balance of the unfertilized treatment. The first would be higher losses by leaching because of a lower uptake of P from deeper layers, or possibly because the productive grass-clover sward in the K fertilized treatment absorbed most of the mobile soil P before it could leach into deeper soil layers. This suggestion is supported by the findings of Dodd et al. (2014), who show that phytoextraction of P by increased productivity of grassland with an N source led to large reductions of leaching. For our experiment, this remains a suggestion because we did not measure leaching and did not calculate leaching using our P concentrations in the topsoil because several authors have shown that this is unsecure (Eriksson et al., 2013; Djodjic et al., 2004) and leaching is field specific. An alternative explanation could be that the measurement of total P did miss some form of soil P into which more P was converted in the treatment without K fertilization, but we did not find a reference to such a phenomenon. Third, reallocation of an amount of P downward in the root system could have differed between treatments because K fertilization led to slight differences in species composition (data not shown). However, it appears unlikely that this mechanism could account for a difference of 59 kg P. Still, this factor would require further investigation.

We conclude that intensively harvested grass-clover fertilized with K is an effective alternative to topsoil removal to reduce excess P in former agricultural soils. Moreover, considering the future shortage of P fertilizers, this method provides an elegant way to recycle P (i.e., to transfer excess soil P from new nature areas back into the agricultural system by cattle feed). The excess P will end up in manure and can then be used on more intensively managed areas where it is needed.

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References

Abelson, P.H. 1999. A potential phosphate crisis. Science 283:2015. doi:10.1126/science.283.5410.2015

Bakker, M., S.J. Alam, J. van Dijk, M. Rounsevell, T. Spek, and A. van den Brink. 2015. The feasibility of implementing an ecological network in The Netherlands under conditions of global change. Landscape Ecol. 30:791–804. doi:10.1007/s10980-014-0145-5

Ball, D.F. 1964. Loss-on-ignition as estimate of organic matter and organic carbon in non-calcareous soils. J. Soil Sci. 15:84–92. doi:10.1111/j.1365-2389.1964.tb00247.x

Barkman, J.J., H. Doing, and S. Segal. 1964. Kritische bemerkungen und vorschläge zur quantitativen vegetationsanalyse. (In German.) Acta Bot. Neerl. 13:394–419. doi:10.1111/j.1438-8677.1964.tb00164.x

Behrendt, H., and A. Boekhold. 1993. Phosphorus saturation in soils and groundwaters. Land Degrad. Rehabil. 4:233–243. doi:10.1002/ldr.3400040406

Bemestingsadvies. 2012. Commissie bemesting grasland en voedergewassen, Postbus 338, 6700 AH Wageningen. www.bemestingsadvies.nl/bemestingsadvies/Adviesbasis%20november%202014.pdf (accessed 25 Oct. 2015).

Bennett, E.M., S.R. Carpenter, and N.F. Caraco. 2001. Human impact on erodable phosphorus and eutrofication: A global perspective. Bioscience 51:227–234. doi:10.1641/0006-3568(2001)051[0227:HIOEPA]2.0.CO;2

Berendse, F., M.J.M. Oomes, H.J. Altena, and W.T. Elberse. 1992. Experiments on the restoration of species-rich meadows in The Netherlands. Biol. Conserv. 62:59–65. doi:10.1016/0006-3207(92)91152-I

Blaser, R.E., and N.C. Brady. 1950. Nutrient competition in plant associations. Agron. J. 42:128–135.

Ceulemans, T., C.J. Stevens, L. Duchateau, H. Jacquemyn, D.J.G. Gowing, R. Merckx, H. Wallace, N. van Rooijen, T. Goethem, R. Bobbink, E. Dorland, C. Gaudnik, D. Alard, E. Corcket, S. Muller, N.B. Dise, C. Dupré, M. Diekmann, and O. Honnay. 2014. Soil phosphorus constrains biodiversity across European grasslands. Glob. Change Biol. 20:3814–3822. doi:10.1111/gcb.12650

Critchley, C.N.R., B.J. Chambers, J.A. Fowbert, A. Bhogal, S.C. Rose, and R.A. Sanderson. 2002. Plant species richness, functional type and soil properties of grasslands and allied vegetations in English environmentally sensitive areas. Grass Forage Sci. 57:82–92. doi:10.1046/j.1365-2494.2002.00305.x

Csathó, P. and L. Radimszky, 2009. Two worlds within EU27: sharp contrasts in organic and mineral nitrogen-phosphorus use, nitrogen-phosphorus balances, and soil phosphorus status: Widening and deepening gap between Western and Central Europe. Commun. Soil Sci. Plan. 40:999–1019.

De Smet, J., G. Hofman, J. Vanderdeelen, M. Van Meirvenne, and L. Baert. 1996. Phosphate enrichment in the sandy loam soils of West-Flanders, Belgium. Fert. Res. 43:209–215. doi:10.1007/BF00747704

Djodjic, F., K. Börling, and L. Bergström. 2004. Phosphorus leaching in relation to soil type and soil phosphorus content. J. Environ. Qual. 33:678–684. doi:10.2134/jeq2004.6780

Dodd, R.J., R.W. McDowell, and L.M. Condron. 2014. Manipulation of fertiliser regimes in phosphorus enriched soils can reduce phosphorus loss to leachate through an increase in pasture and microbial biomass production. Agric. Ecosyst. Environ. 185:65–76. doi:10.1016/j.agee.2013.12.018

Egnér, H., H. Riehm, and W.R. Domingo. 1960. Untersuchungen über die chemische bodenanalyse als grundlage für die beurteilung des nährstoff zustandes der böden: II. Chemische extraktionsmethoden zur phosphor- und kaliumbestimmung. Kungl. Lantbrukshögsk. Annal. 26:199–215.

Eriksson, K., B. Ulén, L. Berzina, A. Iital, V. Janssons, A.S. Sileika, and A. Toomsoo. 2013. Phosphorus in agricultural soils around the Baltic Sea: Comparison of laboratory methods as indices for phosphorus leaching to waters. Soil Use Manage. 29:5–14. doi:10.1111/j.1475-2743.2012.00402.x

Fujita, Y., H. Olde Venterink, P.M. van Bodegom, J.C. Douma, G.W. Heil, N. Hölzel, E. Jablonska, W. Kotowski, T. Okruszko, P. Polikowski, P.C. de Ruiter, and M.J. Wassen. 2014. Low investment in sexual reproduction threatens plants adapted to phosphorus limitation. Nature 505:82–86. doi:10.1038/nature12733

Geurts, J.J.M., P.A.G. van de Wouw, A.J.P. Smolders, J.G.M. Roelofs, and L.P.M. Lamers. 2011. Ecological restoration on former agricultural soils: Feasibility of in situ phosphate fixation as an alternative to top soil removal. Ecol. Eng. 37:1620–1629. doi:10.1016/j.ecoleng.2011.06.038

Gibson, P.B. 1957. Effect of flowering on the persistence of white clover. Agron. J. 49:213–215. doi:10.2134/agronj1957.00021962004900040013x

Gierus, M., J. Kleen, R. Loges, and F. Taube. 2012. Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. Anim. Feed Sci. Technol. 172:150–161. doi:10.1016/j.anifeedsci.2011.12.026

Goodman, P.J., and M. Collison. 1981. Uptake of 32P labelled phosphate by clover and ryegrass growing in mixed swards with different nitrogen treatments. Ann. Appl. Biol. 98:499–506. doi:10.1111/j.1744-7348.1981.tb00782.x

Herring, J.R., and R.J. Fantel. 1993. Phosphate rock demand into the next century: Impact on world food supply. Nat. Resour. Res. 2:226–246. doi:10.1007/BF02257917

Houba, V.J.G., J.J.G. Van der Lee, and I. Novozamsky. 1997. Soil and plant analysis: Part 1. Soil analysis procedures. Wageningen Univ., Wageningen, The Netherlands.

Isermann, K. 1990. Share of agriculture in nitrogen and phosphorus emissions into the surface water of western Europe against the background of their eutrofication. Fert. Res. 26:253–269. doi:10.1007/BF01048764

Janssens, F., A. Peeters, J.R.B. Tallowin, J.P. Bakker, R.M. Bekker, F. Fillat, and M.J.M. Oomes. 1998. Relationship between soil chemical factors and grassland diversity. Plant Soil 202:69–78. doi:10.1023/A:1004389614865

Koopmans, G.F., W.J. Chardon, P. de Willigen, and W.H. van Riemsdijk. 2004a. Phosphorus desorption dynamics in soil and the link to a dynamic concept of bioavailability. J. Environ. Qual. 33:1393–1402. doi:10.2134/jeq2004.1393

Koopmans, G.F., W.J. Chardon, P.A.I. Ehlert, J. Dolfing, R.A.A. Suurs, O. Oenema, and W.H. van Riemsdijk. 2004b. Phosphorus availability for plant uptake in a phosphorus-enriched noncalcareous sandy soil. J. Environ. Qual. 33:965–975. doi:10.2134/jeq2004.0965

Marrs, R.H. 1985. Techniques for reducing soil fertility for nature conservation purposes: A review in relation to research at Roper's Heath, Suffolk, Engeland. Biol. Conserv. 34:307–332. doi:10.1016/0006-3207(85)90038-2

Mengel, K., and D. Steffens. 1985. Potassium uptake of rye-grass (*Lolium perenne*) and red clover (*Trifolium pratense*) as related to root parameters. Biol. Fertil. Soils 1:53–58. doi:10.1007/BF00710971

Ott, C., and H. Rechberger. 2012. The European phosphorus balance. Resour. Conserv. Recycl. 60:159–172. doi:10.1016/j.resconrec.2011.12.007

Piano, E., and P. Annicchiarico. 1995. Persistence of ladino white clover ecotypes and its relationship with other agronomic traits. Grass Forage Sci. 50:195–198. doi:10.1111/j.1365-2494.1995.tb02314.x

Royal Meteorological Institute. 2012. Weather station Gilze-Rijen. www.knmi.nl/nederland-nu/klimatologie/daggegevens (accessed 22 Oct. 2012).

Schoumans, O.F., F. Bouraoui, C. Kabbe, O. Oenema, and C.K. van Dijk. 2015. Phosphorus management in Europe in a changing world. Ambio 44:180–192. doi:10.1007/s13280-014-0613-9

Schoumans, O.F., and W.J. Chardon. 2015. Phosphate saturation degree and accumulation of phosphate in various soil types in The Netherlands. Geoderma 237-238:325–335. doi:10.1016/j.geoderma.2014.08.015

Schwertmann, U. 1964. Diff erenzierung der eisenoxide des bodens durch extraction mit ammoniumoxalat-lösung. Z. Pflanzenernaehr. Dueng. Bodenkd. 105:194–202. doi:10.1002/jpln.3591050303

Sissingh, H.A. 1971. Analytical technique of the Pw method, used for the assessment of the phosphate status of arable soils in the Netherlands. Plant Soil 34:483–486. doi:10.1007/BF01372800

Sival, F.P., and W.J. Chardon. 2004. Nature development on phosphate saturated soils: Phosphate removal by crops. (In Dutch.) Alterra-report 1090. Alterra, Wageningen UR, Wageningen, The Netherlands.

Smolders, A.J.P., E.C.H.E.T. Lucassen, M. van der Aalst, L.P.M. Lamers, and J.G.M. Roelofs. 2008. Decreasing the abundance of *Juncus effusus* on former agricultural lands with noncalcareous sandy soils: Possible effects of liming and soil removal. Restor. Ecol. 16:240–248. doi:10.1111/j.1526-100X.2007.00267.x

Soegaard, K. 2009. Nitrogen fertilization of grass/clover swards under cutting or grazing by dairy cows. Acta Agric. Scand. Sect. B 59:139–150.

Van der Salm, C., W.J. Chardon, G.F. Koopmans, J.C. van Middelkoop, and P.A.I. Ehlert. 2009. Phytoextraction of phosphorus-enriched grassland soils. J. Environ. Qual. 38:751–761. doi:10.2134/jeq2008.0068

Wassen, M.J., H. Olde Venterink, E.D. Lapshina, and F. Tanneberger. 2005. Endangered plants persist under phosphorus limitation. Nature 437:547–550. doi:10.1038/nature03950

Woodfield, D.R., and J.R. Caradus. 1996. Factors affecting white clover persistence in New Zealand pastures. In: Proceedings of the New Zealand Grassland Association 58, Oamaru. 21–24 Oct. 1996. New Zealand Grassland Association, Dunedin, NZ. p. 229–235.

Yli-Halla, M., H. Hartikainene, and P. Väätainen. 2002. Depletion of soil phosphorus as assessed by several indices of phosphorus supplying power. Eur. J. Soil Sci. 53:431–438. doi:10.1046/j.1365-2389.2002.00471.x