Phytomining to re-establish phosphorus-poor soil conditions for nature restoration on former agricultural land

Authors

Schelfhout Stephanie[1,a], De Schrijver An[1,2], Vanhellemont Margot[2], Vangansbeke Pieter[1], Wasof Safaa[1], Michael P Perring[1,3], Haesaert Geert[4], Verheyen Kris[1], Mertens Jan[1]

Affiliations

[1]Forest & Nature Lab, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode (Melle), Belgium.

[2]Faculty of Science and Technology, University College Ghent, Brusselsesteenweg 161, 9090 Melle, Belgium.

[3]Ecosystem Restoration and Intervention Ecology (ERIE) Research Group, School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009 Australia

[4]Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium.

[a]Corresponding author: email Stephanie.Schelfhout@ugent.be; telephone number +3292432489

Keywords

Abiotic ecological restoration, phytoextraction, bioavailable phosphorus, mowing, P-mining, species-rich grassland

Abstract

Aims

To restore species-rich grasslands on former agricultural land, typically phosphorus-poor soil conditions need to be re-established. Here we assess the potential of phosphorus extraction by biomass production, *i.e.* phytomining. We compare two techniques: (i) 'mowing', *i.e.* cutting and removing hay two or three times a year, and (ii) 'P-mining', *i.e.* mowing with yield maximization by adding growth-limiting nutrients other than phosphorus (*i.e.* nitrogen and potassium).

Methods

In a five-year field experiment at three fields situated along a soil phosphorus gradient, we studied phosphorus removal through both biomass assessment and changes in two soil phosphorus pools: bioavailable phosphorus (P_{Olsen}) and slowly cycling phosphorus ($P_{Oxalate}$).

Results

Phosphorus-mining doubled the phosphorus removal with biomass compared to mowing, and phosphorus removal with biomass was lower at fields with an initially lower concentration of P_{Olsen} in the soil. The P_{Olsen} concentrations decreased significantly during the experiment with the largest decreases in phosphorus-rich plots. Changes in the P_{Olsen} and $P_{Oxalate}$ stocks were correlated with the amount of phosphorus removed with biomass.

Conclusions

Phosphorus-mining effectively increases phosphorus removal compared to mowing, but becomes less efficient with decreasing soil phosphorus concentrations. Restoring phosphorus-poor soil conditions on formerly fertilized land remains a challenge: phytomining most often needs a long-term commitment.

Abbreviations

P	Phosphorus
N	Nitrogen
K	Potassium
P_{Olsen}	Sodium bicarbonate-extractable soil P
P_{Oxalate}	Ammonium-oxalate-extractable soil P
P_{AL}	Ammonium-lactate-extractable soil P
P_{Total}	Total soil P
ΔP_{Olsen}	Change in P_{Olsen} between 2011 and 2017
$\Delta P_{\text{Oxalate}}$	Change in P_{Oxalate} between 2011 and 2017
PNI	Phosphorus nutrition index
NNI	Nitrogen nutrition index
KNI	Potassium nutrition index

Introduction

Semi-natural grasslands can be extremely species-rich (Wilson et al. 2012) and are capable of providing a multitude of ecosystem services such as pollination, biological pest control and carbon sequestration (Weisser et al. 2017). Unfortunately, these multifunctional ecosystems are also threatened. For example, 75% of European grassland habitat types are in an unfavourable conservation status and almost 50% are still deteriorating (European Commission 2015). To avoid further losses, it is necessary to conserve remaining habitats and to accelerate ecological restoration actions (Ceballos et al. 2015). Effective restoration requires identification of the threats and ecological mechanisms that can influence successful restoration (Perring et al. 2015), and evidence-based assessments are needed for verifying successful restoration techniques (Suding 2011).

One of the main drivers of the deterioration of species-rich grassland is land-use intensification. Adding fertilizers to increase productivity has strong negative effects on species richness and composition (Gough and Marrs 1990; Walker et al. 2004). As a consequence of fertilization, plant species composition changes from communities with numerous nutrient-limited, slowly growing plant species to communities with just a few fast-growing competitive plant species (Harpole and Tilman 2007; Hautier et al. 2009). Over the past 50 years, nutrients have accumulated in agricultural soils due to repeated fertilization, particularly in areas with a surplus of animal manure (Bouwman et al. 2012; Ringeval et al. 2017). For the restoration of species-rich grasslands, re-establishing nutrient-limiting soil conditions is essential (Marrs 1993; Walker et al. 2004; Wassen et al. 2005; Gilbert et al. 2009). High phosphorus levels in particular do cause species loss (Schellberg and Hejcman 2007; Ceulemans et al. 2014; van Dobben et al. 2017). For restoring phosphorus-limited or co-limited vegetation types such as *Nardus* grasslands (Schelfhout et al. 2017), calcareous grasslands (Niinemets and Kull 2005) and lowland hay meadows (Gilbert et al. 2009), re-establishing phosphorus-poor soil conditions appears to be essential. Yet, it is still unclear how soil phosphorus concentrations can be lowered most effectively and efficiently.

Understanding and predicting changes in soil phosphorus is complicated because of the various inorganic and organic types of phosphorus occurring in the soil (De Schrijver et al. 2012; Kruse et al. 2015), the numerous chemical methods for phosphorus extraction (for an overview of current methods, see Wuenscher et al. 2015) and the different conceptual soil phosphorus models that have been developed (Alvarez and Steinbach 2017). The *Phosphorus Pool Model* defined in Roberts and Johnston (2015) considers inorganic soil phosphorus to reside in four different, inter-related pools that differ in their availability for plant uptake. The first and second pools contain phosphorus in the soil solution and phosphorus that is surface-adsorbed to soil components. These *bioavailable* phosphorus pools are immediately or quite readily available to plants and can be determined through extraction in sodium bicarbonate (P_{Olsen}; Olsen et al. 1954). The third and fourth pool consist of phosphorus that is more strongly bound onto and contained in soil components such as iron and aluminium (hydr)oxides, organic material and clay particles. For the objective of re-establishing phosphorus-poor soil conditions, it is important to not only get insight into the bioavailable phosphorus pools, but also into the phosphorus pools that can feed these bioavailable pools on a timescale of many years (*i.e.* the *slowly cycling* phosphorus pool; Syers et al. 2008; De Schrijver et al. 2012; Johnston et al. 2014). Extraction with ammonium oxalate ($P_{Oxalate}$; Schwertmann 1964) gives a measure of this slowly cycling phosphorus pool (van Rotterdam et al. 2012).

In a field-based experiment, we investigated the effectiveness of two restoration techniques for phosphorus removal and their effect on the concentrations and stocks of bioavailable and slowly cycling phosphorus in the soil. We compared (i) mowing, *i.e.* cutting grassland swards to remove nutrients with hay, and (ii) P-mining, *i.e.* mowing management combined with fertilization of nitrogen and potassium (growth-limiting nutrients other than phosphorus) to maximize biomass production and thus phosphorus removal. Mowing is widely used as a restoration technique on former agricultural land (*e.g.* Bakker et al. 2002; Török et al. 2011; Schelfhout et al. 2017). After several years of mowing, however, the biomass production generally decreases due to limitation by nitrogen and potassium (Van Der Woude et al. 1994; Oelmann et al. 2009), and therefore less phosphorus will be removed with hay. P-mining could be more effective at reducing soil phosphorus concentrations. Yet, only little field evidence on the effects of long-term P-mining is available, especially in comparison with mowing management. Recently, Johnston et al. (2016) compiled several long-term phosphorus depletion experiments and found bioavailable phosphorus to decline exponentially, with the rate of decline depending on the soil's phosphorus-buffering capacity, actual phosphorus saturation and ability to restore the equilibrium between the different phosphorus pools. We performed our experiment from 2011-2016 (*i.e.* during 5 years) in three fields differing in historical land-use intensity and thus representing a soil phosphorus gradient. For each studied field and restoration technique, we assessed the annual biomass yield and phosphorus removed with biomass. We expected (i) to remove more phosphorus by P-mining than by mowing; (ii) to deplete the stocks of bioavailable and slowly cycling phosphorus with a magnitude similar to the amount of phosphorus removed with biomass; and (iii) significant effects of the initial soil phosphorus concentration on (i) and (ii). We discuss the implications of our results in an ecological restoration context.

Materials and methods

Study sites and experimental setup

We selected three fields with comparable soil conditions but different soil phosphorus concentrations to trade space for time, and hence allow for investigating phosphorus removal with biomass while soils become depleted of phosphorus. The study fields lay in Landschap de Liereman, a nature reserve in northern Belgium (Supplementary Fig. S1). The fields were similar with regard to climate, hydrology and soil texture (Table 1), but differed in soil phosphorus concentrations because of differences in historical land-use intensity. The fields were previously managed as fertilized grasslands mown for hay production; fertilization ceased in 1990 for Liereman-1 and in 2005 for Liereman-2 and Liereman-3. The aim of the field owner (non-profit organization Natuurpunt) is to restore species-rich *Nardus* grasslands (European habitat type 6230*), which locally remain in small patches and road verges.

The experiment was set up by sowing the same species-mixture at all fields in April 2011. First, the existing vegetation was removed by glyphosate application and ploughing the 0-30 cm soil layer. Second, the soils were limed with 1424 kg CCE (Calcium Carbonate Equivalent) ha^{-1} (2687 kg granular Dolokorn ha^{-1} containing: > 920 g [$CaCO_3$ + $MgCO_3$] kg^{-1}, from which at least 530 g CaO kg^{-1} and 300 g $MgCO_3$ kg^{-1}; following recommendations by the Belgian Soil Service) and harrowed. Third, we sowed the commercial Herbagreen plus mixture from Philip seeds at a seeding rate of 40 kg ha^{-1} with a seed drill: *Lolium perenne* L. cultivars Plenty (32%), Roy (20%), Milca (15%) and Alcancia (10%); *Phleum pratense* L. subsp. *pratense* cultivar Lirocco (10%); *Festuca pratensis* Huds. cultivar Preval (5%); *Poa pratensis* L. cultivar Balin (3%); and the herb species *Trifolium repens* L. (5%). This seed mixture contains species that can be used for forage production (*i.e.* the produced hay has an economical value to the farmer) on sandy soils. Liming was repeated every two years with 1200 kg CCE ha^{-1} (3000 kg granular Dolokorn ha^{-1} containing: > 600 g $CaCO_3$ kg^{-1} and > 300 g $MgCO_3$ kg^{-1}, from which at least 400 g CaO kg^{-1} and 150 g MgO; following recommendations by the Belgian Soil Service). In May 2012, we applied herbicides to favour the sown grass species (Bofix: 40 g [(4-Amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetic acid L^{-1}, 20 g 3,6-Dichloropyridine-2-carboxylic acid L^{-1} and 200 g (4-Chloro-2-methylphenoxy)acetic acid L^{-1} at a rate of 5 L ha^{-1}). In 2013 and 2014, this was repeated in the parts of the fields managed by P-mining.

At each field, we marked eight plots of 4.5 m by 7 m, which we then further managed (from 2011-2016) by one of the two studied restoration techniques: mowing in four plots, P-mining in the other four plots, according to a randomized block design (Supplementary Fig. S2 and S3). The total number of plots was 24 (8 plots x 3 fields). The mowing plots were managed without fertilization; the P-mining plots with annual fertilization of nitrogen (130 kg N ha^{-1} as NH_4NO_3) and potassium (225 kg K ha^{-1} as $K_2SO_4.MgSO_4$, following recommendations by the Belgian Soil Service). All plots were mown twice a year, except for 2011, *i.e.* the first year, when the vegetation was not fully developed and was mown only once, and 2013 when we mowed three times because of high standing crop biomass in August (see Supplementary Table S1). Each year, two thirds of the total annual amount of fertilizer were applied in March and one third after the first cut. We described the vegetation composition in June 2017 (see Supplementary Table S2).

Biomass sampling and chemical analyses

We sampled each plot two times per year, right before the fields were mown. All plots of a field were sampled (and mown) at the same time (see Supplementary Table S1 for sampling dates). We harvested the biomass in the central part of each plot with a mowing machine equipped with a finger-bar of 1.1 m width along a length of 2 to 4 m, which was measured immediately after cutting. We weighed the fresh biomass with hanging scales in the field, and took a subsample for measuring the dry matter content of the biomass (after drying the sample to constant weight at 65°C for at least 48 h).

The dry subsamples were ground and digested with 0.4 ml $HClO_4$ (65%) and 2 ml HNO_3 (70%) in Teflon bombs at 140°C for 4 h. In these extracts, the phosphorus concentration was measured colorimetrically according to the malachite green procedure (Lajtha et al. 1999) and the potassium concentration was measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS, Agilent, Santa Clara, United States of America). The nitrogen concentration was measured by high-temperature combustion at 1150°C using an elemental analyzer (Vario MACRO cube CNS, Elementar, Hanau, Germany).

Soil sampling and chemical analyses

In April 2011 and January 2017, we collected soil samples with an auger of 3 cm diameter at two depths (0-15 and 15-30 cm) at three locations equally spread in the centre of each plot. We combined the three samples into a mixed sample per plot and per sampling depth, dried them at 40°C for 48 h and passed them through a 2 mm sieve. The bioavailable and slowly cycling phosphorus concentrations were measured by the following two methods: (i) extraction in sodium bicarbonate ($NaHCO_3$; according to ISO 11263:1994(E)) to measure bioavailable phosphorus (P_{Olsen}), which is available for plants within one growing season (Gilbert et al. 2009), and (ii) extraction in ammoniumoxalate-oxalic acid ($(NH_4)_2C_2O_4$ according to NEN 5776:2006) to measure slowly cycling phosphorus ($P_{Oxalate}$), which includes phosphorus that can become available on the longer term (van Rotterdam et al. 2012). In the 2011 samples, also the total phosphorus concentration (P_{Total}) was measured after complete destruction with perchloric acid ($HClO_4$; 65%), nitric acid (HNO_3; 70%) and sulphuric acid (H_2SO_4; 98%) in Teflon bombs at 150°C for 4 h. In the extracts, the phosphorus concentration was measured colorimetrically according to the malachite green procedure (Lajtha et al. 1999).

To measure soil bulk density at the two sampling depths, we collected undisturbed soil samples with standard sharpened steel 100 cm^3 Kopecky rings (diameter 5.1 cm, height 5 cm). We sampled at four locations in the centre of each field (5 sampling locations in Liereman-1 to cover the potential heterogeneity in this field). At each sampling location, we removed the topsoil containing most of the plant roots with a shovel and drove two rings in the 7-12 cm soil layer. We carefully exposed the 15-30 cm soil layer and collected another two rings from the 18-23 cm soil layer. The samples were dried until constant weight at 105°C for 48 h and weighed.

Calculations

We calculated the bulk density of the soil by dividing the dry weight of the soil sample by the volume of the Kopecky ring. Stocks of P_{Olsen} and $P_{Oxalate}$ were calculated by multiplying the bulk density with the phosphorus concentration and depth of the concerned soil layer. We calculated the changes in the P_{Olsen} and $P_{Oxalate}$ concentrations and stocks over time (ΔP_{Olsen}, $\Delta P_{Oxalate}$) by subtracting the 2011 from the 2017 values.

We calculated phosphorus removal with biomass by multiplying the biomass with its phosphorus concentration for each sampling time, and summed them per year to calculate annual biomass and phosphorus removal. We then calculated mean annual biomass and phosphorus removal for the years 2012 until 2016, leaving out 2011 because the vegetation was not yet fully developed then. The mean annual phosphorus concentration in dry biomass was calculated by dividing mean annual phosphorus removal by mean annual biomass.

We derived agronomic nutrient indices for phosphorus, nitrogen and potassium to describe the nutrient limitation for biomass growth according to Duru and Thélier-Huché (1995) and Duru and Ducrocq (1997). We calculated the phosphorus, nitrogen and potassium nutrition indices (PNI, NNI, KNI) as

$$PNI = 100 \times P\%/(0.065 \times N\% + 0.15),$$

$$NNI = 100 \times N\%/(4.8 \times biomass^{-0.32}),$$

$$KNI = 100 \times K\%/(1.6 + 0.525 \times N\%),$$

with *P%*, *N%* and *K%* the percentage of phosphorus, nitrogen and potassium in the *biomass* of the first cut (May-June) and with *biomass* the dry matter content of the biomass in the first cut in t ha^{-1}. Values of 100% indicate there is no limitation by phosphorus, nitrogen or potassium; values above 100% indicate there is luxury consumption of this nutrient; and values below 100% indicate deficiency (Duru and Ducrocq 1997). For instance, values below 60% indicate severe limitation by this nutrient (Mládková et al. 2015).

Statistical analyses

First, to investigate the effects of restoration technique (mowing vs. P-mining) and P_{Olsen} concentration in 2011 ($P_{Olsen-2011}$) on annual biomass properties for the period 2012-2016, we fitted linear mixed effects models with the function *lme* of the "nlme" package using the restricted estimates maximum likelihood method (REML) (Pinheiro et al. 2017). The different annual response variables we considered were: phosphorus removal with biomass, biomass yield (square root transformed), phosphorus concentration in biomass (log transformed), PNI (log transformed), NNI and KNI. The response variables were transformed if necessary to ensure normality of the residuals and homogeneity of variance following Zuur et al. (2009). The full models had the following form: response variable ~ restoration technique X $P_{Olsen-2011}$ + restoration technique X $P_{Olsen-2011}^2$. We used *plot* nested within *field* nested within *year* as a random factor to account for the nested structure of the data and the repeated measures (yearly observations) in a plot. We compared the null model including random effects only, the intermediate and full models based on the corrected Akaike Information Criteria (AICc) and Akaike weights (Zuur et al. 2009) with the *AICctab* function of the "bbmle" package (Bolker and R Core Team 2016). In case of competitive models, *i.e.* multiple models with a difference in AIC_c (ΔAIC_c) of less than two (Goodenough et al. 2012), we used ANOVA tests to select the optimal model with the function *anova* of the "stats" package (R Core Team 2016). For the selected final models, we obtained chi-square (χ^2) values and significance of the fixed terms using Type II Wald chi-square tests with the *anova* function of the "stats" package. We assessed the goodness of fit of the optimal models by calculating the marginal and conditional R^2 (R^2_m and R^2_c) with the *r.squaredGLMM* function of the "MuMIn" package (Bartoń 2018), with R^2_m representing the variance explained by fixed factors and R^2_c the variance explained by both fixed and random factors.

Second, to investigate whether the soil phosphorus concentrations (P_{Olsen} and $P_{Oxalate}$) decreased over the course of the experiment, and whether this decrease was affected by the restoration technique, we fitted linear mixed effects models of the following form: P_{Olsen} or $P_{Oxalate}$ ~ restoration technique X Year, with *field* as random factor. We fitted linear models as we expected the decline in phosphorus concentrations to be linear during the relatively short time of our experiments (*i.e.* 5 years).

Third, we investigated the effects of restoration technique and $P_{Olsen-2011}$ on the changes in P_{Olsen} and $P_{Oxalate}$ concentrations (ΔP_{Olsen}, $\Delta P_{Oxalate}$) at 0-15 cm and 15-30 cm depth with linear mixed effects models of the form: ΔP_{Olsen} or $\Delta P_{Oxalate}$ ~ restoration technique X $P_{Olsen-2011}$. We also evaluated the relation between the $\Delta P_{Oxalate}$ and ΔP_{Olsen} concentrations with a linear mixed effects model of the form: $\Delta P_{Oxalate}$ concentration ~ ΔP_{Olsen} concentration. Finally, the $\Delta P_{Oxalate}$ and ΔP_{Olsen} stocks were compared with the cumulative phosphorus removal with a model of the form: ΔP_{Olsen} or $\Delta P_{Oxalate}$ stock ~ restoration technique X cumulative phosphorus removal. In these three models, we used *field* as random factor.

In the mixed effects models with heterogeneous variances among fields, we allowed for a different variance per field using weights *varIdent* (Pinheiro et al. 2017). Results were considered significant when the *p*-value was less than 0.05 unless indicated otherwise. All statistical analyses were performed with R (R Core Team 2016); graphs were made with the "ggplot" package (Wickham 2009).

Results

Phosphorus removal with biomass

The biomass yield, the phosphorus concentration in the biomass and consequently the annual phosphorus removal were all significantly affected by the applied restoration technique (Fig. 1; Supplementary Fig. S4; Table 2; Supplementary Table S3). Between 2012 and 2016, we annually removed significantly more biomass and more phosphorus from the P-mining plots than from the mowing plots (biomass: 7 ± 2 vs. 3 ± 1 ton dry matter ha^{-1} y^{-1}; phosphorus: 18 ± 7 vs. 8 ± 5 kg P ha^{-1} y^{-1}; $p < 0.001$). The concentration of phosphorus in the biomass, however, was lower in the P-mining plots than in the mowing plots (2.5 ± 0.7 vs. 2.9 ± 0.7 g P kg^{-1}; $p < 0.001$). The biomass yield, the phosphorus concentration in the biomass and the annual phosphorus removal were significantly affected by the concentration of P_{Olsen} in the soil at the beginning of the experiment ($P_{Olsen-2011}$) and, except for the biomass yield, by the interactions between $P_{Olsen-2011}$ and the applied restoration technique (Table 2). The applied restoration technique and $P_{Olsen-2011}$ explained 67% of the variation (R^2_m) in phosphorus removal with biomass. The interannual variation explained a large proportion of the remaining variation (51%): *e.g.* summer droughts, such as in 2014 and 2015, probably caused a drop in the biomass production during the dry months and consequently a drop in the phosphorus removal with biomass.

The agronomic phosphorus nutrient index (PNI) of the biomass in 2012-2016 differed significantly between the two restoration techniques and was quadratically related to the concentration $P_{Olsen-2011}$ in the soil and the interactions between $P_{Olsen-2011}$ and restoration technique ($p < 0.001$ for restoration technique; $p < 0.01$ for $P_{Olsen-2011}$ and the interactions; Supplementary Tables S4 and S5; Supplementary Fig. S5a). Phosphorus was not limiting in any of the mowing plots (mean PNI = 98 ± 21%), but appeared to be limiting in the mining plots of the field with the lowest soil phosphorus concentrations (mean PNI in Liereman-1 = 55 ± 13%). The biomass production in the mowing plots was limited by potassium (mean KNI 36 ± 15%; Supplementary Fig. S5c) and nitrogen (mean NNI 41 ± 13%; Supplementary Fig. S5b), with especially severe potassium limitation in the field Liereman-1 (mean KNI 24 ± 6%). The applied fertilizers in the mining plots resulted in only limited potassium limitation (mean KNI 82 ± 19%) but could not prevent nitrogen limitation (mean NNI 67 ± 17%), which was, however, less severe than in the mowing plots ($p < 0.001$).

Changes in soil phosphorus concentrations

The P_{Olsen} concentration in the 0-15 cm soil layer decreased significantly during the field experiment with on average 14 mg P_{Olsen} kg^{-1} ($p < 0.001$; Supplementary Fig S6a). The 2011-2017 change in P_{Olsen} concentration was larger in the soils with a high initial concentration of P_{Olsen} and larger in the P-mining plots of the Liereman-3 field (Table 3; Supplementary Table S6; Fig. 2a). The P_{Olsen} concentration in 2017 was on average 25% lower than in 2011 and this ratio was apparently lower for the highest initial P_{Olsen} concentrations, however, this effect was not significant (data not shown). We found no significant change in P_{Olsen} concentration in the 15-30 cm soil layer (Supplementary Fig S6c). There was a strong positive correlation between the change in P_{Olsen} concentration and the change in $P_{Oxalate}$ concentration ($p < 0.001$; R^2_m = 76%; Fig. 3; Supplementary Table S7). However, the change in the $P_{Oxalate}$ concentration varied widely in the 0-15 cm soil layer, and we found no significant change in $P_{Oxalate}$ concentrations for either soil layer (Table 3; Supplementary Fig. S6b and Fig. S6d; Fig. 2b).

Phosphorus removal with biomass versus change in phosphorus stocks

There was a weak negative relationship between the 2011-2017 change in the stock of P_{Olsen} in the 0-15 cm soil layer on the one hand and the removal of phosphorus with biomass on the other hand ($p < 0.1$, $R^2_m = 15\%$; Supplementary Tables S8 and S9; Fig. 4a). The relationship between the change in stock of P_{Olsen} in the 0-15 cm soil layer and the restoration technique was also weak ($p < 0.1$, $R^2_m = 13\%$; Supplementary Table S9). In general, the cumulative phosphorus removal with biomass was larger than the change in the stocks of P_{Olsen} in the soil (data points above the 1:1 line in Fig. 4a). The changes of $P_{Oxalate}$ stocks in the 0-15 cm soil layer varied widely and were significantly negatively correlated with the cumulative removal of phosphorus with biomass ($p < 0.05$; $R^2_m = 28\%$; Supplementary Tables S8 and S9; Fig. 4b).

Discussion

Phosphorus removal higher with P-mining and on phosphorus-rich soils

The removal of phosphorus with biomass in the P-mining plots was twice as large as in the mowing plots, and decreased with the initial concentration of P_{Olsen} in the soil. Johnston et al. (2016) also reported high phosphorus removal on phosphorus-rich soils (20-25 kg P ha^{-1}, similar to our results) and found phosphorus removal to be related to the soil phosphorus concentration on soils with less phosphorus. The lower phosphorus removal in the plots containing less P_{Olsen} in our space-for-time field experiment indicates that the extra phosphorus removal gained by P-mining will likely diminish over time during restoration. Hence, when estimating the time needed to restore phosphorus-poor soil conditions through mowing or P-mining, the decrease in annual phosphorus removal after a certain soil phosphorus level has been reached should be taken into account.

The difference in phosphorus removal between P-mining and mowing plots was mainly driven by the higher biomass yield in the P-mining plots, seeing that the phosphorus concentration in the biomass was lower in the P-mining plots. The swards in the P-mining plots yielded, on average, two times more biomass than in the mowing plots. Our mining swards annually yielded 7 ± 2 t dry matter ha^{-1}, in line with other fertilized grasslands (on average 6 t dry matter ha^{-1} y^{-1} in Duffková et al. 2015) but lower than the intensively fertilized grasslands that produced up to 14 t dry matter ha^{-1} y^{-1} in Liebisch et al. (2013). The yields in our mowing plots, *i.e.* 3 ± 1 t dry matter ha^{-1} y^{-1}, were similar to the yields in grasslands under nature management mown for 15 to 25 years in Belgium and the Netherlands (Bakker et al. 2002; Schelfhout et al. 2017). In the swards under mowing regime, the biomass production was severely limited by nitrogen and potassium from the second growing season onwards, in line with the well-described nitrogen and potassium depletion of soils through leaching or plant uptake (Van Der Woude et al. 1994; Smits et al. 2008; Oelmann et al. 2009; Storkey et al. 2015). The restraint on biomass production due to nitrogen and potassium limitation in the mowing plots probably explains that the biomass yield in these plots only weakly increased with soil phosphorus concentrations (Table 2). Soil phosphorus appeared to be limiting only in the P-mining plots of the field with the lowest soil phosphorus concentration (Liereman-1; indicated by PNI < 60% *cf*. Mládková et al. 2015; Supplementary Fig. S5a).

The phosphorus concentration in the harvested biomass was affected by both the initial concentration of P_{Olsen} in the soil and the applied restoration technique. The plant phosphorus concentrations were positively correlated with the P_{Olsen} concentrations in the soil, *e.g.* in the P-mining plots we saw a twofold increase from on average 1.8 ± 0.3 mg P g^{-1} in the phosphorus-poor field (Liereman-1) to on average 3.0 ± 0.1 mg P g^{-1} in the phosphorus-rich field (Liereman-3; Supplementary Fig. S4b). In the Liereman-3 plots, the average PNI for June was equal or greater than 100%, which indicates no limitation by phosphorus or even *luxury consumption* of this nutrient (Duru and Ducrocq 1997), *i.e.* an increase in phosphorus uptake with soil phosphorus concentration that does not result in additional biomass (Aerts and Chapin 1999). Interestingly, this luxury consumption of phosphorus also occurred in other P-mining studies: two pot experiments (Schelfhout et al. 2015; Schelfhout et al. 2018) and a long-term field study (Bauke et al. 2018). The phosphorus concentration in the biomass was lower in the P-mining plots than in the mowing plots, which may be caused by: (i) *dilution*, *i.e.* concentrations of nutrients in plants tend to decrease - become diluted - as the plants produce more biomass (Hejcman et al. 2010), or (ii) *(temporary) phosphorus limitation* in the rhizosphere (Hinsinger 2001) as a result of a lag in the replenishment of the pools of bioavailable phosphorus in the soil, formerly described as *transactional phosphorus limitation* in Vitousek et al. (2010).

Decreasing P_{Olsen} over time

In the majority of the plots, the concentration of P_{Olsen} in the 0-15 cm soil layer was 25% lower at the end of the experiment than before the experiment started. In absolute numbers, the drop appeared to be larger in phosphorus-rich plots. We found a clear distinction in phosphorus removal with biomass between the two applied restoration techniques, and the overall 2011-2016 phosphorus removal with biomass was related to the change in P_{Olsen} and $P_{Oxalate}$ stocks in the 0-15 cm soil layer. Changes in bioavailable soil phosphorus concentrations can be caused by plant phosphorus uptake through roots, translocation to shoots and subsequent removal with hay, but can also happen through phosphorus fixation in roots, uptake by microbial biomass or accumulation in soil organic matter. These organic pools of soil phosphorus can be a *sink* (*e.g.* incorporation of phosphorus into living microbial biomass), but can (later) also become a *source* of bioavailable phosphorus through mineralization and release of microbial phosphorus after cell death (von Lützow et al. 2006). Inorganic phosphorus can be fixed by migration into organic phosphorus pools and occluded mineral pools (Van der Salm et al. 2017), but phosphorus can also be released from organic or inorganic recalcitrant phosphorus pools. To gain full insight into the complex phosphorus cycle, a thorough fractionation procedure should be executed (cfr. De Schrijver et al. 2012), which was beyond the scope of our study.

We want to note that our results are only valid for soils with a comparable sandy texture, soil acidity and organic matter content. The P_{Olsen} to $P_{Oxalate}$ ratio and the changes in this ratio after phytoextraction of phosphorus can differ across soil types (Cross and Schlesinger 1995; Johnston et al. 2016; Bauke et al. 2018).

Implications for ecological restoration

Phytomining is an effective technique for removing phosphorus from soils: it has a large capability for phosphorus removal, *i.e.* up to 34 kg P ha^{-1} y^{-1} at phosphorus-rich soils. When considering phytomining to re-establish phosphorus-poor soil conditions, managers should however be aware of four drawbacks.

First, on phosphorus-rich fields, large amounts of phosphorus need to be removed to restore phosphorus-poor soil conditions, and this can take several decades (Schelfhout et al. 2017). For the depletion of bioavailable phosphorus pools, it is necessary to also reduce the slowly cycling phosphorus pools that replenish the bioavailable pools. To estimate the restoration potential of specific fields for re-establishing phosphorus-poor soil conditions, practitioners therefore need to assess both the bioavailable and slowly cycling phosphorus pools.

Secondly, the available time and financial budget should match with the choice for a restoration technique despite the law of diminishing returns (see Kubanek 2017 on optimal decision making). The law of diminishing returns impedes the use of phytomining for restoration (Marrs 1993): phosphorus removal with biomass declines with decreasing bioavailable phosphorus concentrations in the soil despite constant restoration efforts. This decrease in phosphorus removal efficiency has to be taken into account when estimating the restoration time needed with phytomining. As we showed here, the potential to remove phosphorus with biomass by either mowing or P-mining did differ depending on the initial soil phosphorus concentration. The advantage of P-mining over mowing (*i.e.* more phosphorus removal through mining) was smaller when the soils contained less bioavailable (< 50 mg P_{Olsen} kg^{-1}) and slowly cycling phosphorus.

A third drawback to consider is that neither the bioavailable nor the slowly cycling phosphorus concentrations in the deeper soil layer (15-30 cm) had been depleted after five years of phytomining (contrary to the significant decline in the bioavailable phosphorus concentrations in the upper 0-15 cm soil layer). Yet, the phosphorus pools in the 15-30 cm soil layer and even the subsoil layer (> 30 cm deep) are likely to be accessible by plant roots or mycorrhiza in symbiosis with these plants, especially with P-mining (Bauke et al 2018). The phosphorus pools in these deeper soil layers should thus be taken into consideration when estimating the restoration potential of a field.

The fourth drawback specifically for P-mining is that fertilization with nitrogen can have negative effects on the surrounding environment if the fertilizer is administered incorrectly. When the applied nitrogen doses are excessive, residual soil mineral nitrogen can leach to the ground water (D'Haene et al. 2014). The risks of using nitrogen fertilizers can be minimized by following fertilizer recommendations to limit off-site loss. Reducing the temporarily high soil nitrogen and potassium concentrations after P-mining is possible by mowing without fertilizer input for a few years (Smits et al. 2008).

Restoring phosphorus-poor soil conditions (*e.g.* <12 mg P_{Olsen} kg^{-1} for restoration of *Nardus* grasslands; Schelfhout et al. 2017) on phosphorus-rich soils, such as the Liereman-3 field in our study, via P-mining or mowing is a time-consuming approach that will take many decades (see estimation of the duration in Schelfhout et al. (2017)). For these parcels, more drastic measures such as mechanically removing the phosphorus-enriched soil layer (*i.e.* topsoil removal; cf. Török et al 2011) can be considered. When reducing the bioavailable and slowly cycling phosphorus pools is not feasible, it might be more appropriate to aim for floristically diverse mesotrophic plant communities that require less phosphorus-poor soil conditions, and certainly are interesting for diverse insect communities that benefit *e.g.* pollination and natural pest control in the surrounding (Woodcock et al. 2014). To work towards this aim, mowing to obtain nitrogen-, potassium- or co-limitation of these nutrients within a few years can be combined with the active introduction of common nonendangered legume and forb species following sward disturbance to enhance their germination (Klaus et al. 2018).

Conclusions

The potential to phytomine phosphorus (with either P-mining or mowing) depends on the phosphorus concentration in the soil, with high phosphorus removal possible on phosphorus-rich soils and the removal potential becoming lower on soils with lower phosphorus concentrations. Phosphorus mining led to larger phosphorus removal with biomass compared to conventional mowing management. It is clear that restoring phosphorus-poor soil conditions on formerly fertilized land will remain a challenge and that phytomining phosphorus is always a long-term commitment. For initially phosphorus-rich parcels, turning the nature restoration goal towards communities of mesotrophic habitats might be more realistic when drastic measures such as removal of the phosphorus-rich topsoil are not an option.

Acknowledgements

We are grateful for the admission to conduct our field experiment in Landschap De Liereman (Natuurpunt): the cooperation with the people of Natuurpunt and local farmers was greatly appreciated. Special thanks to Kris Van der Steen, Jan Van den Berghe and Dieter Dijckmans. Further, we thank our lab technicians Luc Willems and Greet de Bruyn for processing and analysing our samples. Kris Ceunen, Filip Ceunen, Luc Willems, Robbe De Beelde, Jelle Van den Berghe, Danny Vereecke and Tomohiro Nagata are thanked for helping with fieldwork. Lander Baeten and Haben Blondeel are thanked for helping out with the statistics. We thank Rob Marrs, Tobias Ceulemans, Frank Nevens, Lander Baeten and three anonymous reviewers for their helpful comments.

References

Aerts R, Chapin FS (1999) The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. Adv. Ecol. Res. pp 1–67

Alvarez R, Steinbach HS (2017) Modeling soil test phosphorus changes under fertilized and unfertilized managements using artificial neural networks. Agron J 109:2278–2290. doi: 10.2134/agronj2017.01.0014

Bakker JP, Elzinga JA, de Vries Y (2002) Effects of long-term cutting in a grassland system: perspectives for restoration of plant communities on nutrient-poor soils. Appl Veg Sci 5:107–120. doi: 10.1111/j.1654-109X.2002.tb00540.x

Bartoń K (2018) MuMIn: Multi-Model Inference. R package version 1.42.1 https://CRAN.R-project.org/package=MuMIn

Bauke SL, von Sperber C, Tamburini F, et al. (2018) Subsoil phosphorus is affected by fertilization regime in long-term agricultural experimental trials. Eur J Soil Sci 69:103–112. doi: 10.1111/ejss.12516

Bolker B, R Core Team (2016) bbmle: Tools for General Maximum Likelihood Estimation. R package version 1.0.20 https://CRAN.R-project.org/package=bbmle

Bouwman L, Goldewijk KK, Van Der Hoek KW, et al. (2012) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900-2050 period. Proc Natl Acad Sci U S A 110:20882–20887. doi: 10.1073/pnas.1012878108

Ceballos G, Ehrlich PR, Barnosky AD, et al. (2015) Accelerated modern human-induced species losses: Entering the sixth mass extinction. Sci Adv 1:e1400253–e1400253. doi: 10.1126/sciadv.1400253

Ceulemans T, Stevens CJ, Duchateau L, et al. (2014) Soil phosphorus constrains biodiversity across European grasslands. Glob Chang Biol 20:3814–3822. doi: 10.1111/gcb.12650

Cross AF, Schlesinger WH (1995) A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. Geoderma 64:197–214. doi: 10.1016/0016-7061(94)00023-4

De Schrijver A, Vesterdal L, Hansen K, et al. (2012) Four decades of post-agricultural forest development have caused major redistributions of soil phosphorus fractions. Oecologia 169:221–34. doi: 10.1007/s00442-011-2185-8

D'Haene K, Salomez J, De Neve S, et al. (2014) Environmental performance of nitrogen fertiliser limits imposed by the EU Nitrates Directive. Agric Ecosyst Environ 192:67–79. doi: 10.1016/j.agee.2014.03.049

Duffková R, Hejcman M, Libichová H (2015) Effect of cattle slurry on soil and herbage chemical properties, yield, nutrient balance and plant species composition of moderately dry Arrhenatherion grassland. Agric Ecosyst Environ 213:281–289. doi: 10.1016/j.agee.2015.07.018

Duru M, Ducrocq H (1997) A nitrogen and phosphorus herbage nutrient index as a tool for assessing the effect of N and P supply on the dry matter yield of permanent pastures. Nutr Cycl Agroecosystems 47:59–69.

Duru M, Thélier-Huché L (1995) N and P–K status of herbages: use for diagnosis of grasslands. Diagnostic Proced Crop N Manag 125–138.

Egnér H, Riehm H, Domingo W (1960) Untersuchung über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor und Kaliumbestimmung. K Lantbrukshogskolans Ann 26:199–215.

European Commission (2015) Report from the commission to the council and European parliament. The State of Nature in the European Union. Report on the status of and trends for habitat types and species covered by the Birds and Habitats Directives for the 2007-2012 period as required. Brussels, Belgium

Gilbert J, Gowing D, Wallace H (2009) Available soil phosphorus in semi-natural grasslands: Assessment methods and community tolerances. Biol Conserv 142:1074–1083. doi:

10.1016/j.biocon.2009.01.018

Goodenough AE, Hart AG, Stafford R (2012) Regression with empirical variable selection: Description of a new method and application to ecological datasets. PLoS One 7:1–10. doi: 10.1371/journal.pone.0034338

Gough MW, Marrs RH (1990) A Comparison of Soil Fertility between Semi-Natural and Agricultural Plant-Communities - Implications for the Creation of Species-Rich Grassland on Abandoned Agricultural Land. Biol Conserv 51:83–96.

Harpole WS, Tilman D (2007) Grassland species loss resulting from reduced niche dimension. Nature 446:791–3. doi: 10.1038/nature05684

Hautier Y, Niklaus PA, Hector A (2009) Competition for Light Causes Plant Biodiversity Loss After Eutrophication. Science 324:636–638. doi: 10.1126/science.1169640

Hejcman M, Szaková J, Schellsberg J, Tlustoš P (2010) The Rengen Grassland Experiment: relationship between soil and biomass chemical properties, amount of elements applied, and their uptake. Plant Soil 333:163–179. doi: 10.1007/s11104-010-0332-3

Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemcial changes: a review. Plant Soil 237:173–195.

Johnston AE, Poulton PR, Fixen PE, Curtin D (2014) Phosphorus. Its Efficient Use in Agriculture., 1st ed. Adv Agron. doi: 10.1016/B978-0-12-420225-2.00005-4

Johnston AE, Poulton PR, White RP, Macdonald AJ (2016) Determining the longer term decline in plant-available soil phosphorus from short-term measured values. Soil Use Manag 32:151–161. doi: 10.1111/sum.12253

Klaus VH, Hoever CJ, Fischer M, et al. (2018) Contribution of the soil seed bank to the restoration of temperate grasslands by mechanical sward disturbance. Restor Ecol 26:114–122. doi:

10.1111/rec.12626

Kruse J, Abraham M, Amelung W, et al. (2015) Innovative methods in soil phosphorus research : A review. J Plant Nutr Soil Sci 178:43–88. doi: 10.1002/jpln.201400327

Kubanek J (2017) Optimal decision making and matching are tied through diminishing returns. Proc Natl Acad Sci 114:8499–8504. doi: 10.1073/pnas.1703440114

Lajtha K, Driscoll CT, Jarrell WM, Elliott ET (1999) Soil Phosphorus: Characterization and Total Element Analysis. In: Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds) Stand. Soil Methods Long-Term Ecol. Res. Oxford University Press, New York, pp 115–142

Liebisch F, Bünemann EK, Huguenin-Elie O, et al. (2013) Plant phosphorus nutrition indicators evaluated in agricultural grasslands managed at different intensities. Eur J Agron 44:67–77. doi: 10.1016/j.eja.2012.08.004

Marrs RH (1993) Soil Fertility and Nature Conservation in Europe: Theoretical Considerations and Practical Management Solutions. Adv Ecol Res 24:241–300. doi: 10.1016/S0065-2504(08)60044-6

Mládková P, Mládek J, Hejduk S, et al. (2015) High-nature-value grasslands have the capacity to cope with nutrient impoverishment induced by mowing and livestock grazing. J Appl Ecol 52:1073–1081. doi: 10.1111/1365-2664.12464

Niinemets Ü, Kull K (2005) Co-limitation of plant primary productivity by nitrogen and phosphorus in a species-rich wooded meadow on calcareous soils. Acta Oecologica 28:345–356. doi: 10.1016/j.actao.2005.06.003

Oelmann Y, Broll G, Holzel N, et al. (2009) Nutrient impoverishment and limitation of productivity after 20 years of conservation management in wet grasslands of north-western Germany. Biol Conserv 142:2941–2948. doi: 10.1016/j.biocon.2009.07.021

Olsen SR, Cole C V, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ 939.

Perring MP, Standish RJ, Price JN, et al. (2015) Advances in restoration ecology: rising to the challenges of the coming decades. Ecosphere 6:1–25. doi: 10.1890/ES15-00121.1

Pinheiro J, Bates D, Debroy S, et al. (2017) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131 https://CRAN.R-project.org/package=nlme

R Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Ringeval B, Augusto L, Monod H, et al. (2017) Phosphorus in agricultural soils: drivers of its distribution at the global scale. Glob Chang Biol 23:3418–3432. doi: 10.1111/gcb.13618

Roberts TL, Johnston AE (2015) Phosphorus use efficiency and management in agriculture. Resour Conserv Recycl 105:275–281. doi: 10.1016/j.resconrec.2015.09.013

Schelfhout S, De Schrijver A, De Bolle S, et al. (2015) Phosphorus mining for ecological restoration on former agricultural land. Restor Ecol 23:842–851. doi: 10.1111/rec.12264

Schelfhout S, De Schrijver A, Verheyen K, et al. (2018) Phosphorus mining efficiency declines with decreasing soil P concentration and varies across crop species. Int J Phytoremediation 20:939–946. doi: 10.1080/15226514.2018.1448363

Schelfhout S, Mertens J, Perring MP, et al. (2017) P-removal for restoration of Nardus grasslands on former agricultural land: cutting traditions. Restor Ecol 25:S178–S187. doi: 10.1111/rec.12531

Schellberg J, Hejcman M (2007) The Rengen Grassland Experiment (1941 - 2006) and its contribution to grassland ecology. Grassl Sci Eur 12:512–515.

Schwertmann U (1964) Differenzierung der Eisenoxide des Bodens durch Extraktion mit

Ammoniumoxalat-Lösung. J Plant Nutr Soil Sci 105:194–202.

Smits N a. C, Willems JH, Bobbink R (2008) Long-term after-effects of fertilisation on the restoration of calcareous grasslands. Appl Veg Sci 11:279–286. doi: 10.3170/2008-7-18417

Storkey J, Macdonald AJ, Poulton PR, et al. (2015) Grassland biodiversity bounces back from long-term nitrogen addition. Nature 528:401–404. doi: 10.1038/nature16444

Suding KN (2011) Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. Annu Rev Ecol Evol Syst 42:465–487. doi: 10.1146/annurev-ecolsys-102710-145115

Syers JK, Johnston AE, Curtin D (2008) Efficiency of Soil and Fertilizer Phosphorus Use: Reconciling Changing Concepts of Soil Phosphorus Behaviour with Agronomic Information. FAO Fertil Plant Nutr Bull. doi: 10.1017/S0014479708007138

Török P, Vida E, Deák B, et al. (2011) Grassland restoration on former croplands in Europe: An assessment of applicability of techniques and costs. Biodivers Conserv 20:2311–2332. doi: 10.1007/s10531-011-9992-4

van der Salm C, van Middelkoop JC, Ehlert PAI (2017) Changes in soil phosphorus pools of grasslands following 17 yrs of balanced application of manure and fertilizer. Soil Use Manag 33:2–12. doi: 10.1111/sum.12333

Van Der Woude BJ, Pegtel DM, Bakker JP (1994) Nutrient Limitation After Long-Term Nitrogen Fertilizer Application in Cut Grasslands. J Appl Ecol 31:405–412.

van Dobben HF, Wamelink GWW, Slim PA, et al. (2017) Species-rich grassland can persist under nitrogen-rich but phosphorus-limited conditions. Plant Soil 411:451–466. doi: 10.1007/s11104-016-3021-z

van Rotterdam AMD, Bussink DW, Temminghoff EJM, van Riemsdijk WH (2012) Predicting the potential of soils to supply phosphorus by integrating soil chemical processes and standard soil tests. Geoderma 189–190:617–626. doi: 10.1016/j.geoderma.2012.07.003

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecol Appl 20:5–15.

von Lützow M, Kögel-Knabner I, Ekschmitt K, et al. (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. Eur J Soil Sci 57:426–445. doi: 10.1111/j.1365-2389.2006.00809.x

Walker KJ, Stevens P a, Stevens DP, et al. (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. Biol Conserv 119:1–18. doi: 10.1016/j.biocon.2003.10.020

Walkley A, Black IA (1934) An examination of the Djegtareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci 37:29–38.

Wassen MJ, Venterink HO, Lapshina ED, Tanneberger F (2005) Endangered plants persist under phosphorus limitation. Nature 437:547–550. doi: 10.1038/nature03950

Weisser WW, Roscher C, Meyer S, et al. (2017) Biodiversity effects on ecosystem functioning in a 14-year grassland experiment : patterns , mechanisms , and open questions. Basic Appl Ecol 23:1–73. doi: http://dx.doi.org/10.1016/j.baae.2017.06.002

Wickham H (2009) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Wilson JB, Peet RK, Dengler J, Pärtel M (2012) Plant species richness: the world records. J Veg Sci 23:796–802. doi: 10.1111/j.1654-1103.2012.01400.x

Woodcock BA, Savage J, Bullock JM, et al. (2014) Enhancing floral resources for pollinators in productive agricultural grasslands. Biol Conserv 171:44–51. doi: 10.1016/j.biocon.2014.01.023

Wuenscher R, Unterfrauner H, Peticzka R, Zehetner F (2015) A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. Plant Soil Environ 61:86–96. doi: 10.17221/932/2014-PSE

Zuur A, Ieno E, Walker N, et al. (2009) Mixed effects models and extensions in ecology with R. Springer, New York

Tables

Table 1 Initial soil properties of the three fields (2011). Samples from the 0-30 cm soil layer consisted of six soil cores bulked together to one sample per field. Samples from the 0-15 and 15-30 cm soil layers are shown by mean ± standard deviation with eight samples per field (*i.e.* one bulked sample for each plot); each of the samples consisted of three soil cores. Bulk density is shown by mean ± standard deviation with eight samples at four locations per field

	Depth (cm)	**Liereman-1**	**Liereman-2**	**Liereman-3**
Coordinates		51°20’0.1”N 5°1’1.7”E	51°19’58.4”N 5°0’57.4”E	51°20’50.5”N 5°1’11.2”E
Township		Oud-Turnhout	Oud-Turnhout	Oud-Turnhout
Nature reserve		Landschap De Liereman	Landschap De Liereman	Landschap De Liereman
Mean annnual precipitation[a] (mm)		870	870	870
Mean annual temperature[a] (°C)		10.5	10.5	10.5
BEMEX[b] classification	**0-30**	Coarse sand	Coarse sand	Coarse sand
WRB[c] classification	**0-30**	Gleyic Podzol (Arenic)	Albic Podzol (Arenic)	Gleyic Podzol (Arenic)
Organic C (weight %)[d]	**0-30**	4.3	1.5	1.4
pH_{KCl} [d]	**0-30**	4.1	4.8	4.6
P_{AL}(mg kg^{-1})[d]	**0-30**	30	130	300
K_{AL} (mg kg^{-1})[d]	**0-30**	30	<20	40
Ca_{AL} (mg kg^{-1})[d]	**0-30**	380	290	300
Mg_{AL} (mg kg^{-1})[d]	**0-30**	40	40	50
P_{Olsen} (mg kg^{-1})	**0-15**	29 ± 17	71 ± 16	112 ± 16
	15-30	27 ± 21	70 ± 15	108 ± 21
$P_{Oxalate}$ (mg kg^{-1})	**0-15**	66 ± 32	220 ± 84	422 ± 34[e]
	15-30	71 ± 47	225 ± 94	417 ± 52[e]
P_{Total} (mg kg^{-1})	**0-15**	196 ± 62	366 ± 118	605 ± 113
	15-30	245 ± 115	372 ± 116	565 ± 119
Bulk density (g cm^{-3})	**0-15**	1.5 ± 0.2	1.6 ± 0.1	1.5 ± 0.1
	15-30	1.5 ± 0.2	1.6 ± 0.1	1.5 ± 0.1

[a]Data between 1981 and 2010 by the Royal Meteorological Institute of Belgium for Oud-Turnhout; [b]Soil classification according to Belgian Soil Service: “Coarse sand”: sandy fraction [50 µm - 2 mm] > 90% and median of sandy fraction > 200 µm and clay fraction [< 2 µm] < 8%; [c]Soil classification according to the World Reference Base for Soil Resources classification (WRB); [d]To characterize these fields, one bulked sample (regular grid, auger diameter = 3 cm, 6 soil cores per field) of the 0-30 cm soil layer per field was analysed by the lab of the Soil Service of Belgium: soil texture by manual characterization; organic carbon according to an SSB-adjusted Walkley and Black method (Walkley and Black 1934); P, K, Mg and Ca extracted in acid ammonium-lactate (resp. P_{AL}, K_{AL}, Mg_{AL} and Ca_{AL}; Egnér et al. 1960) measured with Inductively Coupled Plasma (ICP); [e]Instead of eight samples, only six samples were analysed for $P_{Oxalate}$ in 2011

Table 2 The optimal models for annual biomass yield (t ha^{-1}), mean phosphorus concentration in the biomass (mg kg^{-1}) and phosphorus removal with biomass (kg ha^{-1}), for 2012-2016, including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{\text{Olsen-2011}}$)[a]

Model	Estimate	Standard error	χ^2	df	*p*-value[b]	R^2_m [c]	R^2_c [c]
Biomass[d]				1,103		67%	97%
Intercept	1.5	0.1	403		***		
Restoration technique	1.0	0.06	340		***		
$P_{\text{Olsen-2011}}$	0.002	0.001	5		*		
P concentration in biomass[e]				1,101		46%	80%
Intercept	0.6	0.09	291		***		
Restoration technique	-0.3	0.06	33		***		
$P_{\text{Olsen-2011}}$	0.01	0.002	80		***		
$P_{\text{Olsen-2011}}{}^2$	-0.00005	0.00001	23		***		
Restoration technique X $P_{\text{Olsen-2011}}$	0.002	0.0007	6		*		
Phosphorus removal with biomass				1,102		66%	96%
Intercept	4.8	1.6	81		***		
Restoration technique	5.0	1.2	193		***		
$P_{\text{Olsen-2011}}$	0.05	0.01	61		***		
Restoration technique X $P_{\text{Olsen-2011}}$	0.06	0.02	11		**		

[a] The full models had the following form: response variable ~ restoration technique X $P_{\text{Olsen-2011}}$ + restoration technique X $P_{\text{Olsen-2011}}{}^2$. We used *plot* nested within *field* nested within *year* as random factors (see Supplementary Table S3 and Fig. S4); [b] Significance of effects is indicated by *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; [c] The percentage of variance explained by the fixed effects (R^2_m) and the full model, including the random effects year, field and plot (R^2_c) are shown for each model; [d] Biomass was square root transformed; [e] Phosphorus concentration in biomass was log transformed

Table 3 The optimal models for the change in P_{Olsen} and P_{Oxalate} concentrations (mg kg^{-1}) between 2011 and 2017 in the 0-15 cm soil layer, including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{\text{Olsen-2011}}$)[a]

Model	Estimate	Standard error	χ^2	df	*p*-value[b]	R^2_m [c]	R^2_c [c]
Change in P_{Olsen} concentration				1,18		40%	88%
Intercept	-28	18	1		*ns*		
Restoration technique	0.5	0.09	68		***		
$P_{\text{Olsen-2011}}$	-10	8	44		***		
Restoration technique X $P_{\text{Olsen-2011}}$	0.3	0.08	10		**		
Change in P_{Oxalate} concentration				1,19		0%	0%
Intercept	7	10	0.4		ns		

[a] The models had the following form: ΔP_{Olsen} or $\Delta P_{\text{Oxalate}}$ ~ restoration technique X $P_{\text{Olsen-2011}}$. We used *field* as a random factor (see Supplementary Table S6); [b] Significance of effects is indicated by *** $p < 0.001$; ** $p < 0.01$; *ns*, not significant; [c] The percentage of variance explained by the fixed effects (R^2_m) and the full model, including the random effects field and plot (R^2_c) are shown for each model

Figures

Fig. 1 The mean and standard deviation of annual removal of phosphorus with biomass (kg ha^{-1} y^{-1}) plotted versus $P_{Olsen-2011}$ (mg kg^{-1}) for the 24 plots with mowing and P-mining treatment in the four study fields. The regression lines show the optimal linear mixed effects model fitted to the 120 data points for the 2012-2016 period (See Table 2 and Supplementary Table S3)

Fig. 2 Changes in P_{Olsen} (a; mg kg^{-1}; n = 24) and $P_{Oxalate}$ (b; mg kg^{-1}; n = 22 because of two missing samples) in the 0-15 cm soil layer between 2011 and 2017 plotted versus $P_{Olsen-2011}$ (mg kg^{-1}). Negative values represent decreases in phosphorus concentrations; positive values show increases in phosphorus concentrations. The line was fitted according to the optimal linear mixed effects model for a transformed change in P_{Olsen} concentration as response variable (See Table 3 and Supplementary Table S6)

Fig. 3 Changes in P_{Olsen} versus P_{Ox} concentrations (mg kg^{-1}) in the 0-15 cm soil layer between 2011 and 2017 (n = 22). Negative values represent decreases in phosphorus concentrations; positive values show increases in phosphorus concentrations. The full black line was fitted according to the linear mixed effects model for change in $P_{Oxalate}$ concentration as response variable (Supplementary Table S7)

Fig. 4 Change in P_{Olsen} stock (a; kg ha^{-1}; n = 24) and $P_{Oxalate}$ stock (b; kg ha^{-1}; n = 22) in the 0-15 cm soil layer between 2011 and 2017 plotted versus the cumulative P removal with biomass (kg ha^{-1}) between 2011 and 2016. Negative values represent decreases in phosphorus stocks; positive values show increases in phosphorus stocks. The dotted line is the 1:1 line. According to the linear mixed effects models, the dashed black line indicates a marginally significant relation ($p < 0.1$) and the full black line indicates a significant relation (See Supplementary Tables S8 and S9)

Supplementary material

Fig. S1 Map of Europe and northern Belgium (magnification in square) with the nature reserve (township between brackets) where the experiment took place. Soil classification according to the Belgian Soil Service

Fig. S2 Example of the experimental setup at each field. We sampled biomass and soil in the central zone within each of the mowing (green) and P-mining (grey) plots. Bulk density measurements were performed on four samples at each field

Fig. S3 Illustration of the effects of the restoration techniques "mowing" and "P-mining" in May 2016 in Liereman-1

Fig. S4 The mean and standard deviation of annual biomass (a; ton ha^{-1} y^{-1}) and annual phosphorus concentration in biomass (b; g kg^{-1}) plotted versus $P_{Olsen-2011}$ (mg kg^{-1}) for the 24 plots with mowing and P-mining treatment in the four study fields. The regression lines show the optimal linear mixed effects model fitted to the 120 data points for the 2012-2016 period

Fig. S5 The nutrient induces for phosphorus (PNI; a), nitrogen (NNI; b) and potassium (KNI; c) in biomass harvested in May or June plotted versus $P_{Olsen-2011}$ (mg kg^{-1}) for the 24 plots. Mean and standard deviation for 2012-2016 are shown. The regression lines show the optimal linear mixed effects model fitted to the 120 data points

Fig. S6 The concentration of P_{Olsen} (a, c; n=24) and $P_{Oxalate}$ (b,d; n=22) in the 0-15 cm (a, b) and 15-30 cm (c, d) soil layers of the mowing and P-mining plots before and after the experiment (2011, 2017). Significance of effects is indicated by *** $p < 0.001$; ns means the difference was not significant

Table S1 Biomass sampling dates for the three fields

Table S2 Vegetation composition in July 2017 in mowing and P-mining plots: mean cover in %; minimal and maximal values are shown between brackets. Cover by grass species is indicated by "G"; cover by forb species by "F" and cover by moss species "M"

Table S3. Model selection of the relationships between annual biomass properties in 2012-2016 and the restoration technique and soil phosphorus concentration at the start of the experiment ($P_{Olsen-2011}$)[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Table S4. Model selection of the relationships between the nutrient indices for phosphorus (PNI), nitrogen (NNI) and potassium (KNI), including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{Olsen-2011}$)[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Table S5 The optimal models for nutrient indices for phosphorus (PNI), nitrogen (NNI) and potassium (KNI) in May-June for 2012-2016, including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{Olsen-2011}$)[a]

Table S6. Model selection of the relationships between the change in P_{Olsen} and $P_{Oxalate}$ concentrations between 2011 and 2017 in the 0-15 cm and 15-30 cm soil layers, including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{Olsen-2011}$)[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Table S7. The model for the change in P_{Olsen} versus P_{Ox} concentration (mg kg^{-1}) in the 0-15 cm soil layer between 2011 and 2017 (n = 22)[a]

Table S8. Model selection of the relationships between the change in P_{Olsen} and $P_{Oxalate}$ concentrations between 2011 and 2017 in the 0-15 cm soil layer, including the restoration technique treatment and the cumulative phosphorus removal with biomass[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Table S9 The optimal models for the change in P_{Olsen} and $P_{Oxalate}$ stocks (kg ha^{-1}) between 2011 and 2017 in the 0-15 cm soil layer, including the restoration technique treatment and cumulative phosphorus removal with biomass[a]

Supplementary material

Phytomining to re-establish phosphorus-poor soil conditions for nature restoration on former agricultural land

Schelfhout Stephanie[1,a], De Schrijver An[1,2], Vanhellemont Margot[1,2], Vangansbeke Pieter[1], Wasof Safaa[1], Michael P Perring[1,3], Haesaert Geert[4], Verheyen Kris[1], Mertens Jan[1]

[1]Forest & Nature Lab, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode (Melle), Belgium.
[2]Faculty of Science and Technology, University College Ghent, Brusselsesteenweg 161, 9090 Melle, Belgium.
[3]Ecosystem Restoration and Intervention Ecology (ERIE) Research Group, School of Plant Biology, The University of Western Australia, 35 Stirling Highway, Crawley WA 6009 Australia
[4]Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium.

[a]Corresponding author: email Stephanie.Schelfhout@ugent.be; telephone number +3292432489
CORRESPONDING AUTHOR: Stephanie Schelfhout. E-mail: Stephanie.Schelfhout@ugent.be

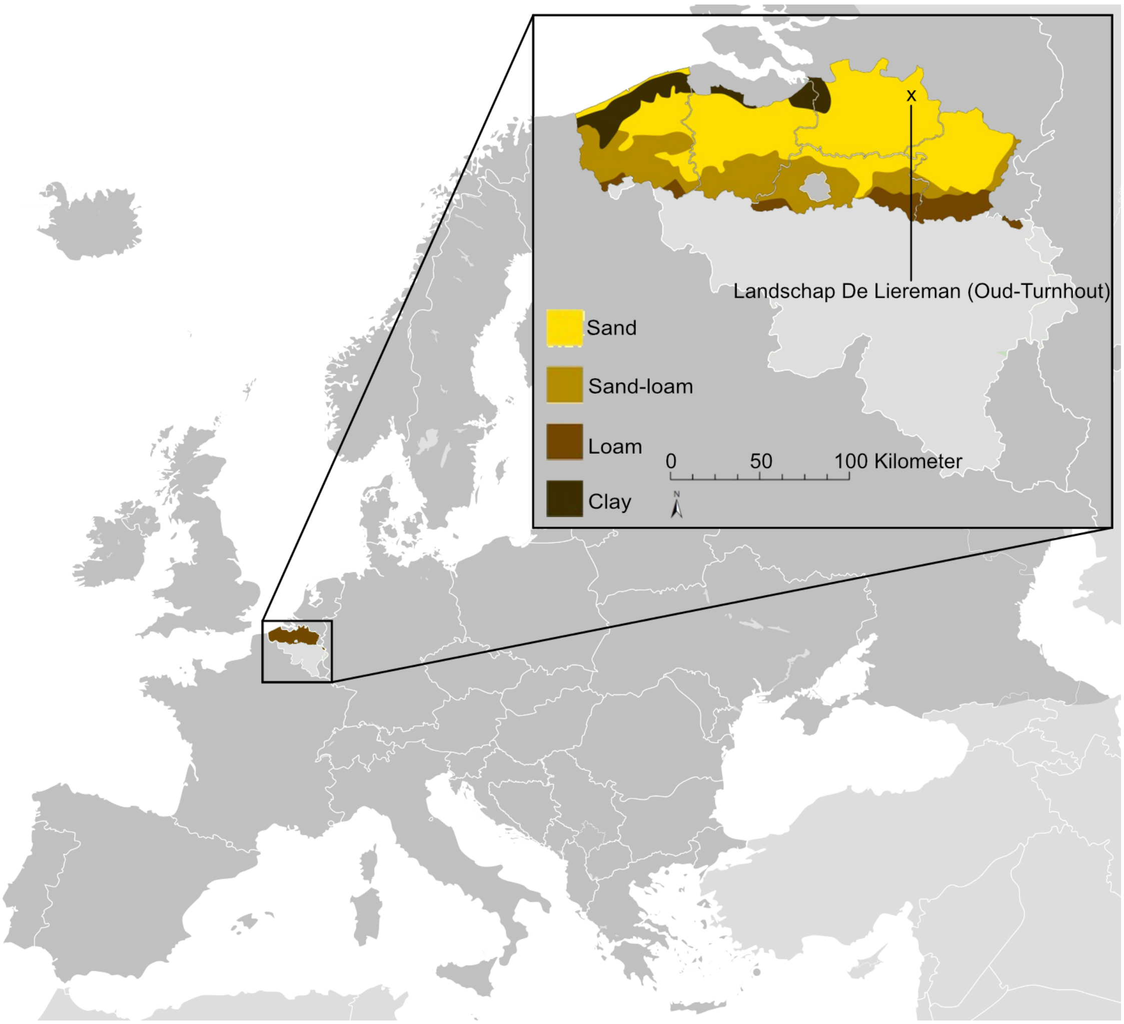

Fig. S1 Map of Europe and northern Belgium (magnification in square) with the nature reserve (township between brackets) where the experiment took place. Soil classification according to the Belgian Soil Service

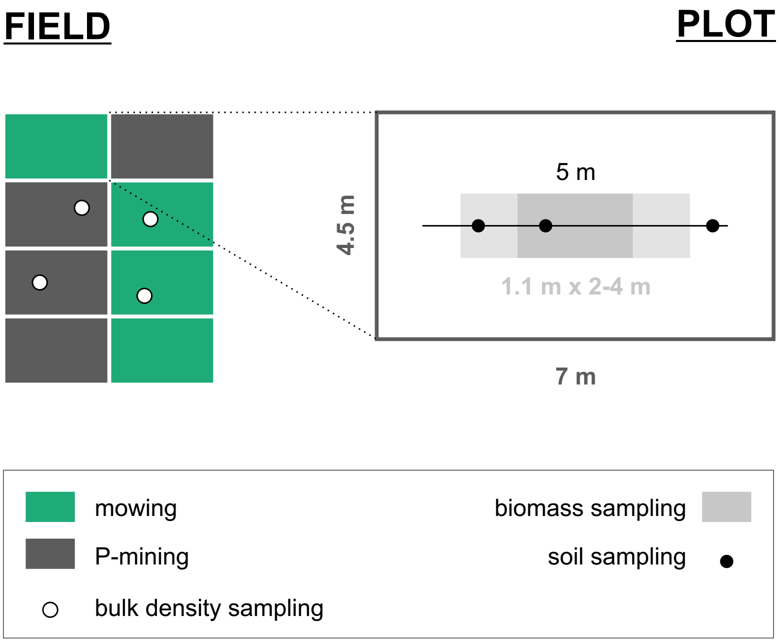

Fig. S2 Example of the experimental setup at each field. We sampled biomass and soil in the central zone within each of the mowing (green) and P-mining (grey) plots. Bulk density measurements were performed on four samples at each field

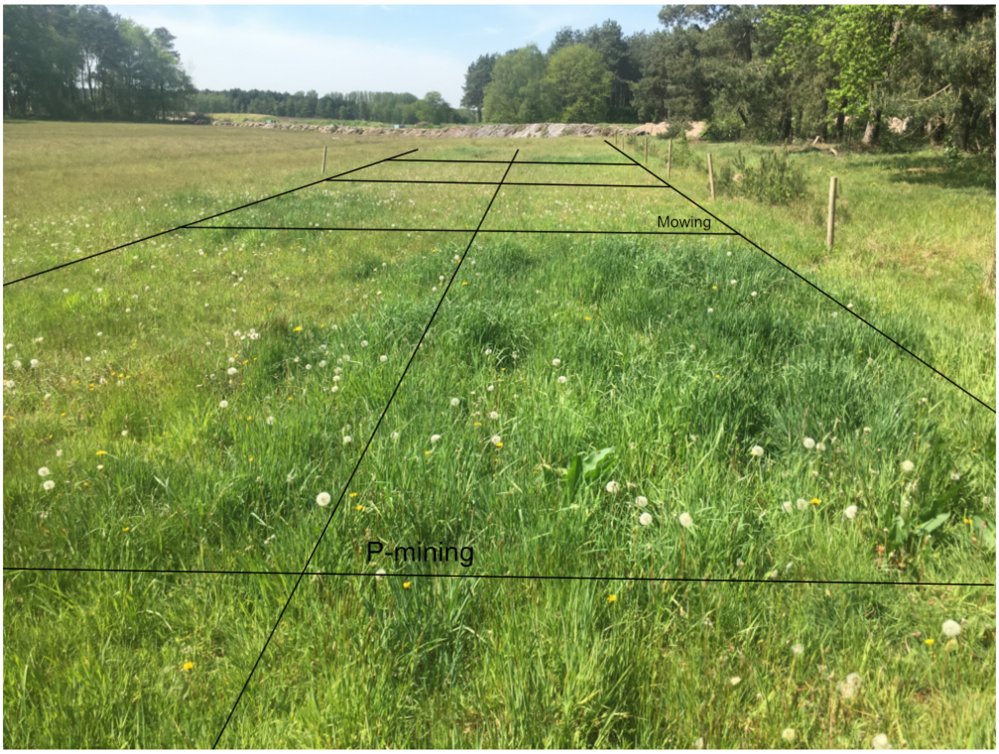

Fig. S3 Illustration of the effects of the restoration techniques "mowing" and "P-mining" in May 2016 in Liereman-1

Table S1 Biomass sampling dates for the three fields

Year	**Harvest**	**Liereman**
2011	1	02/08
2012	1	15/05
	2	21/09
2013	1	25/05
	2	13/08
	3	23/09
2014	1	12/06
	2	10/10
2015	1	21/05
	2	22/10
2016	1	29/06
	2	21/10

Table S2 Vegetation composition in July 2017 in mowing and P-mining plots: mean cover in %; minimal and maximal values are shown between brackets. Cover by grass species is indicated by "G"; cover by forb species by "F" and cover by moss species "M"

	Species	Type	Occurrence in Seed mix (%)	Mowing (%)	P-mining (%)
Sown species-mix in 2011	*Festuca pratensis*	G	5	0.4 (0-5)	0.4 (0-5)
	Lolium perenne	G	77	0.2 (0-1)	9 (0-80)
	Phleum pratense	G	10	16 (0-40)	51 (5-90)
	Poa pratensis	G	3	0	0
	Trifolium repens	F	5	2 (0-10)	4 (0-25)
Spontaneous species found in 2017	*Achillea millefolium*	F		2 (0-25)	0
	Agrostis capillaris	G		36 (0-50)	18 (0-55)
	Agrostis stolonifera	G		9 (0-50)	9 (0-35)
	Anthoxanthum odoratum	G		10 (0-40)	3 (0-25)
	Arrhenatherum elatius	G		0	1 (0-15)
	Betula pendula	F		0.2 (0-1)	0
	Bromus hordeaceus	G		0.1 (0-1)	0
	Cardamine pratensis	F		0.1 (0-1)	0.4 (0-5)
	Carex ovalis	G		0.9 (0-5)	0.7 (0-7)
	Cerastium fontanum	F		7 (1-25)	3 (0-20)
	Cirsium arvense	F		0.5 (0-5)	11 (0-55)
	Cirsium palustre	F		0.7 (0-7)	1 (0-10)
	Conyza canadensis	F		0	0.1 (0-1)
	Crepis capillaris	F		2 (0-7)	0.5 (0-5)
	Dactylis glomerata	G		0	4 (0-30)
	Holcus lanatus	G		28 (10-60)	35 (10-60)
	Jacobaea vulgaris	F		5 (0-40)	3 (0-20)
	Juncus bufonius	G		0.8 (0-10)	0
	Juncus effusus	G		2 (0-10)	0.1 (0-1)
	Leontodon autumnalis	F		7 (0-20)	2 (0-15)
	Lotus pedunculatus	F		4 (0-25)	1 (0-7)
	Luzula campestris	F		3 (0-25)	0.1 (0-1)
	Pinus sylvestris	F		0.1 (0-1)	0
	Plantago lanceolata	F		0	0.1 (0-1)
	Plantago major	F		0.1 (0-1)	0
	Quercus robur	F		0.1 (0-1)	0
	Ranunculus repens	F		21 (0-50)	8 (0-20)
	Rhytidiadelphus squarrosus	M		19 (0-90)	0
	Rumex acetosa	F		0	0.3 (0-1)
	Rumex acetosella	F		7 (0-30)	0.2 (0-1)
	Rumex obtusifolius	F		0	1 (0-7)
	Salix caprea	F		0.2 (0-1)	0
	Sonchus oleraceus	F		1 (0-7)	0
	Taraxacum officinale	F		5 (0-25)	7 (0-20)
	Trifolium dubium	F		0.1 (0-1)	0
	Urtica dioica	F		0.1 (0-1)	2 (0-25)
	Veronica arvensis	F		0.1 (0-1)	0.2 (0-1)

Table S3. Model selection of the relationships between annual biomass properties in 2012-2016 and the restoration technique and soil phosphorus concentration at the start of the experiment ($P_{Olsen\text{-}2011}$)[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Response Variable	Predictor Variables in Model	df	AIC_c	ΔAIC_c	w[b]	*p*-value[c]
Phosphorus removal with biomass	Restoration technique + $P_{Olsen\text{-}2011}$ + Restoration technique : $P_{Olsen\text{-}2011}$ + $P_{Olsen\text{-}2011}^2$ + Restoration technique X $P_{Olsen\text{-}2011}^2$	12	687	0	0.40	*ns*
	Restoration technique + $P_{Olsen\text{-}2011}$ + Restoration technique X $P_{Olsen\text{-}2011}$ + $P_{Olsen\text{-}2011}^2$	11	688	0.3	0.34	
	Restoration technique + $P_{Olsen\text{-}2011}$ + Restoration technique X $P_{Olsen\text{-}2011}$	**10**	**688**	**1.0**	**0.24**	
Biomass[d]	**Restoration technique + $P_{Olsen\text{-}2011}$**	**9**	**96**	**0**	**0.48**	*
	Restoration technique	8	98	1.7	0.21	
Phosphorus concentration in biomass[e]	**Restoration technique + $P_{Olsen\text{-}2011}$ + Restoration technique X $P_{Olsen\text{-}2011}$ + $P_{Olsen\text{-}2011}^2$**	**11**	**-94**	**0**	**0.59**	*ns*
	Restoration technique + $P_{Olsen\text{-}2011}$ + Restoration technique X $P_{Olsen\text{-}2011}$ + $P_{Olsen\text{-}2011}^2$ + Restoration technique X $P_{Olsen\text{-}2011}^2$	12	-93	1.4	0.29	

[a] The full models had the following form: response variable ~ restoration technique X $P_{Olsen\text{-}2011}$ + restoration technique X $P_{Olsen\text{-}2011}^2$. We used *plot* nested within *field* nested within *year* as random factors

[b] *w*: the Akaike weight indicates the probability that the model is the best model of the set of models tested

[c] * indicates a significant difference with $p < 0.05$; *ns* indicates no significant difference between the models

[d] Biomass was square root transformed

[e] Phosphorus concentration in biomass was log transformed

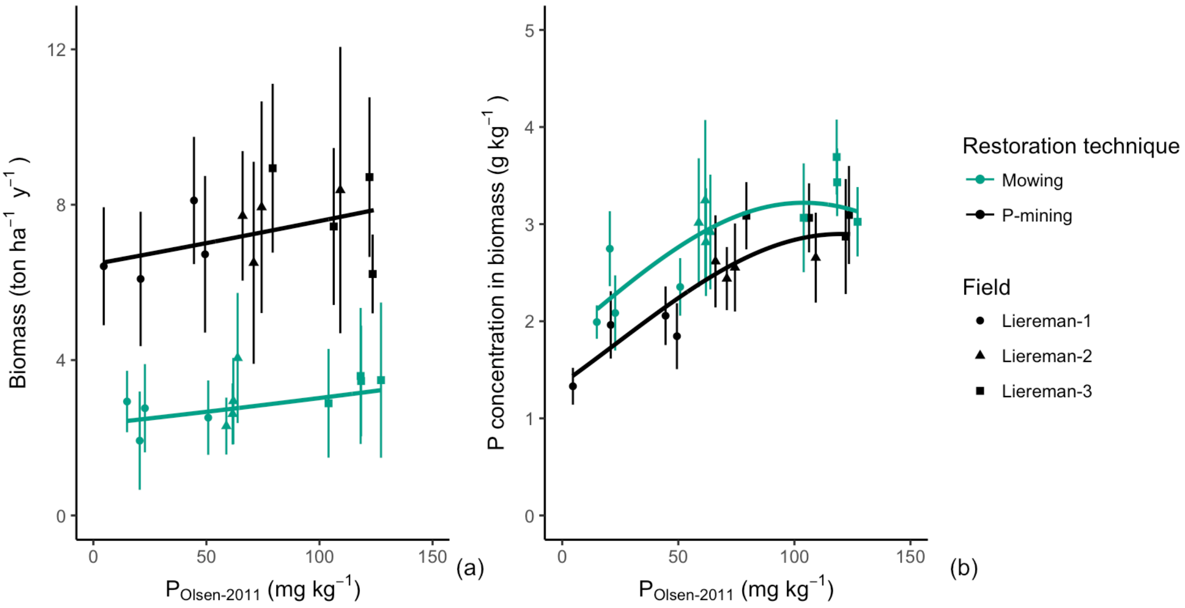

Fig. S4 The mean and standard deviation of annual biomass (a; ton ha^{-1} y^{-1}) and annual phosphorus concentration in biomass (b; g kg^{-1}) plotted versus $P_{Olsen-2011}$ (mg kg^{-1}) for the 24 plots with mowing and P-mining treatment in the four study fields. The regression lines show the optimal linear mixed effect model fitted to the 120 data points for the 2012-2016 period

Table S4. Model selection of the relationships between the nutrient indices for phosphorus (PNI), nitrogen (NNI) and potassium (KNI), including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{Olsen-2011}$)[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Response Variable	Predictor Variables in Model	df	AIC_c	ΔAIC_c	w[b]	*p*-value[c]
PNI[d]						
	Restoration technique + $P_{Olsen-2011}$ + Restoration technique X $P_{Olsen-2011}$ + $P_{Olsen-2011}^2$ + Restoration technique X $P_{Olsen-2011}^2$	**12**	**-123**	**0**	**0.87**	
NNI						
	Restoration technique + $P_{Olsen-2011}$	9	842	0	0.35	*ns*
	Restoration technique	**8**	**842**	**0.7**	**0.26**	
KNI						
	Restoration technique + $P_{Olsen-2011}$ + Restoration technique X $P_{Olsen-2011}$ + $P_{Olsen-2011}^2$	**11**	**884**	**0**	**0.74**	

[a] The full models had the following form: response variable ~ restoration technique X $P_{Olsen-2011}$ + restoration technique X $P_{Olsen-2011}^2$. We used *plot* nested within *field* nested within *year* as random factors

[b] *w*: the Akaike weight indicates the probability that the model is the best model of the set of models tested

[c] *ns* indicates no significant difference between the models

[d] PNI was log transformed

Table S5 The optimal models for nutrient indices for phosphorus (PNI), nitrogen (NNI) and potassium (KNI) in May-June for 2012-2016, including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{Olsen\text{-}2011}$)[a]

Model		**Estimate**	**Standard error**	χ^2	**df**	***p-value***[b]	R^2_m [c]	R^2_c [c]
PNI[d]					1,87		63%	75%
	Intercept	4	0.09	16809		***		
	Restoration technique	-0.6	0.1	62		***		
	$P_{Olsen\text{-}2011}$	0.006	0.002	105		***		
	$P_{Olsen\text{-}2011}{}^2$	-0.00002	0.00001	16		***		
	Restoration technique X $P_{Olsen\text{-}2011}$	0.009	0.003	16		***		
	Restoration technique X $P_{Olsen\text{-}2011}{}^2$	-0.00005	0.00002	7		**		
NNI					1,91		47%	96%
	Intercept	41	5	131		***		
	Restoration technique	25	2	171		***		
KNI					1,88		83%	99%
	Intercept	9	5	258		***		
	Restoration technique	71	4	600		***		
	$P_{Olsen\text{-}2011}$	0.7	0.1	3		.		
	$P_{Olsen\text{-}2011}{}^2$	-0.004	0.0009	17		***		
	Restoration technique X $P_{Olsen\text{-}2011}$	-0.3	0.06	24		***		

[a] The full models had the following form: response variable ~ restoration technique X $P_{Olsen\text{-}2011}$ + restoration technique X $P_{Olsen\text{-}2011}{}^2$. We used *plot* nested within *field* nested within *year* as random factors

[b] Significance of effects is indicated by *** $p < 0.001$; ** $p < 0.01$; . $p < 0.1$.

[c] The percentage of variance explained by the fixed effects (R^2_m) and the full model, including the random effects year, field and plot (R^2_c) are shown for each model

[d] PNI was log transformed

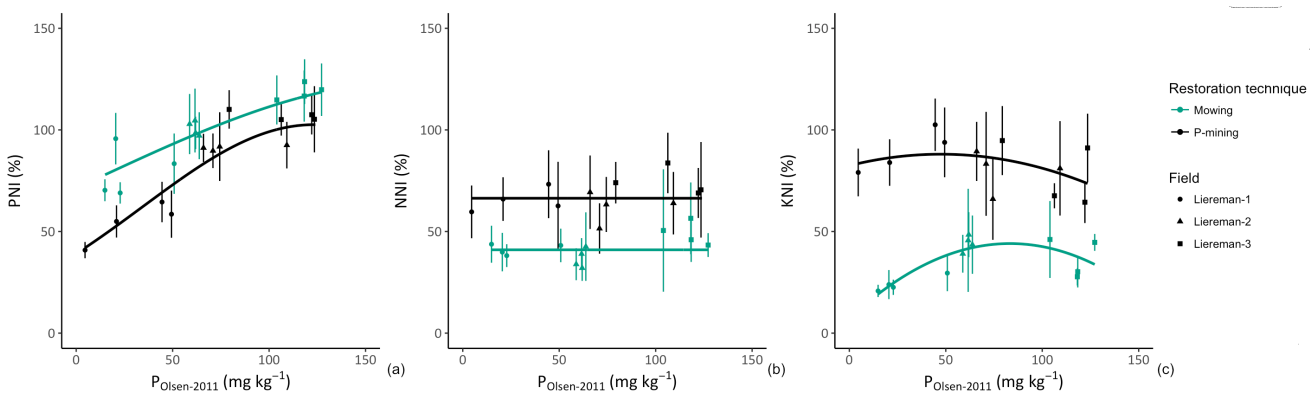

Fig. S5 The nutrient induces for phosphorus (PNI; a), nitrogen (NNI; b) and potassium (KNI; c) in biomass harvested in May or June plotted versus $P_{Olsen\text{-}2011}$ (mg kg^{-1}) for the 24 plots. Mean and standard deviation for 2012-2016 are shown. The regression lines show the optimal linear mixed effect model fitted to the 120 data points

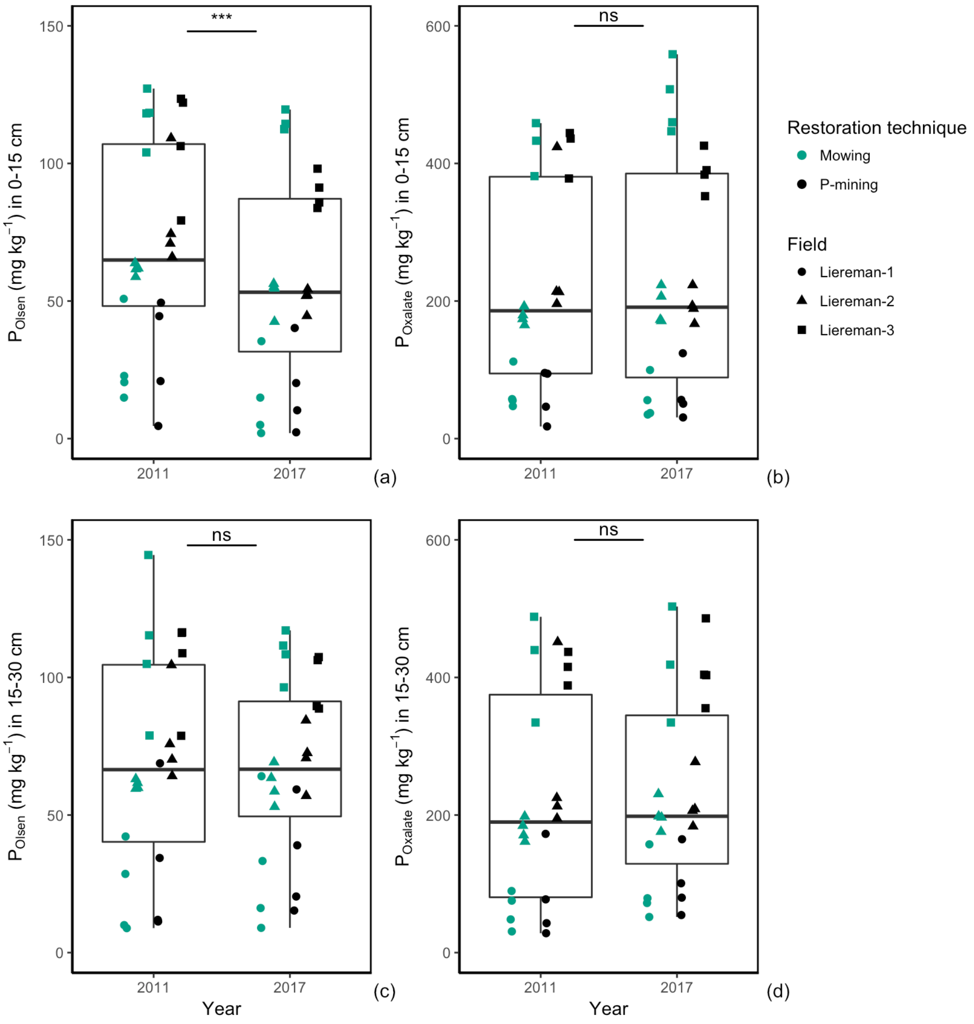

Fig. S6 The concentration of P_{Olsen} (a, c; n=48) and $P_{Oxalate}$ (b,d; n=46) in the 0-15 cm (a, b) and 15-30 cm (c, d) soil layers of the mowing and P-mining plots before and after the experiment (2011, 2017). Significance of effects is indicated by *** $p < 0.001$; ns means the difference was not significant

Table S6. Model selection of the relationships between the change in P_{Olsen} and $P_{Oxalate}$ concentrations between 2011 and 2017 in the 0-15 cm and 15-30 cm soil layers, including the restoration technique treatment and the soil phosphorus concentration at the start of the experiment ($P_{Olsen\text{-}2011}$)[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Response Variable	Predictor Variables in Model	df	AIC_c	ΔAIC_c	*w*[b]
Change in 0-15 cm P_{Olsen} concentration	**Restoration technique + $P_{Olsen\text{-}2011}$ + Restoration technique X $P_{Olsen\text{-}2011}$**	**8**	**198**	**0**	**0.83**
Change in 0-15 cm $P_{Oxalate}$ concentration	**null model**	**5**	**250**	**0**	**0.51**
Change in 15-30 cm P_{Olsen} concentration	**$P_{Olsen\text{-}2011}$**	**6**	**203**	**0**	**0.75**
Change in 15-30 cm $P_{Oxalate}$ concentration	**$P_{Olsen\text{-}2011}$**	**6**	**243**	**0**	**0.69**

[a] The models had the following form: ΔP_{Olsen} or $\Delta P_{Oxalate}$ ~ restoration technique X $P_{Olsen\text{-}2011}$. We used *field* as a random factor

[b] *w*: the Akaike weight indicates the probability that the model is the best model of the set of models tested

Table S7. The model for the change in P_{Olsen} versus P_{Ox} concentration (mg kg^{-1}) in the 0-15 cm soil layer between 2011 and 2017 (n = 22)[a]

Model	Estimate	Standard error	χ^2	df	*p*-value[b]	R^2_m[c]	R^2_c[c]
Change in $P_{Oxalate}$ concentration				1,18		76%	76%
Intercept	-39	9	3		*ns*		
Change in P_{Olsen} concentration	3	0.4	66		***		

[a] The model had the following form: $\Delta P_{Oxalate}$ ~ ΔP_{Olsen}. We used *field* as a random factor

[b] Significant effects are indicated by *** $p < 0.001$. *ns* indicates no significant difference between the models

[c] The percentage of variance explained by the fixed effects (R^2_m) and the full model, including the random effects field and plot (R^2_c) are shown for each model

Table S8. Model selection of the relationships between the change in P_{Olsen} and $P_{Oxalate}$ concentrations between 2011 and 2017 in the 0-15 cm soil layer, including the restoration technique treatment and the cumulative phosphorus removal with biomass[a]. Only competitive models with ΔAIC_c of less than two are shown (Burnham and Anderson, 2002). In case of multiple suitable models according to ΔAIC_c, models were compared with ANOVA tests. The selected optimal models are shown in bold

Response Variable	Predictor Variables in Model	df	AIC_c	ΔAIC_c	w[b]	p-value[c]
Change in P_{Olsen} stock	**Restoration technique**	**4**	**245**	**0**	**0.37**	*ns* .
	Cumulative P removal	**4**	**245**	**0.4**	**0.29**	.
	null model	3	246	0.8	0.24	
Change in $P_{Oxalate}$ stock	**Cumulative P removal**	**4**	**282**	**0**	**0.54**	

[a] The models had the following form: ΔP_{Olsen} or $\Delta P_{Oxalate}$ ~ restoration technique X cumulative P removal. We used *field* as a random factor

[b] *w*: the Akaike weight indicates the probability that the model is the best model of the set of models tested

[c] Significant effects are indicated by . $p < 0.1$. *ns* indicates no significant difference between the models

Table S9. The optimal models for the change in P_{Olsen} and $P_{Oxalate}$ stocks (kg ha^{-1}) between 2011 and 2017 in the 0-15 cm soil layer, including the restoration technique treatment and cumulative phosphorus removal with biomass[a]

Model	Estimate	Standard error	χ^2	df	p-value[b]	R^2_m[c]	R^2_c[c]
Change in P_{Olsen} stock				1,20		15%	24%
Intercept	-1.2	19	12		**		
cumulative $P_{removal}$	0.44	0.2	4		.		
Change in P_{Olsen} stock				1,20		13%	15%
Intercept	19	10	20		***		
Restoration technique	26	14	4		.		
Change in $P_{Oxalate}$ stock				1,18		28%	33%
Intercept	-155	71	0.6		*ns*		
cumulative $P_{removal}$	2.5	0.9	8		*		

[a] The models had the following form: ΔP_{Olsen} or $\Delta P_{Oxalate}$ ~ restoration technique x cumulative P removal. We used *field* as a random factor

[b] Significant effects are indicated by ** $p < 0.01$; * $p < 0.05$; $p < 0.1$. *ns* indicates no significant difference between the models

[c] The percentage of variance explained by the fixed effects (R^2_m) and the full model, including the random effects field and plot (R^2_c) are shown for each model

References

Burnham K, Anderson D (2002) Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, Second edition. Springer-Verlag, New York (USA)