Title: Phosphorus puts a mortgage on restoration of species-rich grasslands on former agricultural land

Running head: Phosphorus puts mortgage on grassland restoration

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Abstract. Large-scale biodiversity loss is one of the most urgent global issues. The Convention on Biological Diversity created a vision to ecologically restore ecosystems by 2050. The European Union follows this ambition, and member states are required to select Special Areas of Conservation (SACs) to develop and restore; one example is species-rich semi-natural grassland. Species-rich grassland restoration requires time for restoring both abiotic conditions, *e.g.* low soil phosphorus concentrations, and biotic conditions, *e.g.* introduction of missing species. For 507 grasslands (in northern Belgium) situated in SACs, we calculated the time needed for restoring necessary phosphorus-poor conditions. Only eleven percent of the grasslands already met the strictest phosphorus-target. We found that less than a fourth of the other 452 grasslands will reach this phosphorus-target by 2050 through mowing. P-mining, a more intensive technique involving fertilization of nitrogen and potassium, could help achieve this phosphorus-target on about slightly more than a third of these grasslands will require alternatives like topsoil removal or selection of a different, less ambitious grassland target. These calculations do not include the time needed for biotic

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restoration. Because grassland restoration is a long-term process, we advocate better protection of still existing species-rich grasslands. Restoration is a last resort in safe-guarding biodiversity.

Keywords: Abiotic restoration, biodiversity decline, Natura 2000, phosphorus mining, semi-natural grasslands, traditional haymaking

Implications for practice:

- When selecting new fields for restoration, soil phosphorus fractions and iron concentrations should be measured beforehand to get an insight for restoration potential. Using this information, practitioners can then decide which target habitat can be reached on which field using which measure.
- Although restoration projects are of utmost importance, primary focus should be on the conservation and protection of remaining species-rich grasslands due to the very long mowing and P-mining times required for abiotic restoration after intensive fertilization.
- To our knowledge, no similar predictive models have been made, although many other countries than Belgium have similar intensive agricultural histories. Therefore, we argue that many other European countries might be facing similar restoration obstacles to reach the EU objectives before 2050.

Introduction

Species-rich grasslands were once widespread all over Europe (Poschlod et al. 2009; Hejcman et al. 2013; Dengler et al. 2014). Because of the low soil nutrient availability and management practices such as haymaking and grazing, a world-record breaking number of plant species can occur on small spatial scales in these grasslands (Wilson et al. 2012). Since the second half of the 20th century, management of these species-rich grasslands was often intensified by increased fertilization, pesticide use and drainage (Green 1990; Muller et al. 1998; Willems 2001), resulting in an overall loss of biodiversity (Grime 2001; Hautier et al. 2009). In order to counter this loss of biodiversity, global engagements were made during the 10th meeting of the Conference of the Parties to the Convention on Biological Diversity to ecologically restore our ecosystems by 2050 (COP Decision X/2 2010; CBD/COP/14/9 2018). Having a pioneering role, the European Union assimilated this 2050 vision into their Environment Action Programme to 2020 (Decision NO 1386/2013/EU 2013). Subsequently, member

states set up Natura 2000 Special Areas of Conservation (SACs) and drew up conservation objectives to develop and restore species-rich grasslands by 2050. Target grassland types had already been appointed for restoration by the European Union (Habitats Directive 92/43/EEC). The UN decade on ecosystem restoration is more ambitious and aims at restoration by 2030 (https://www.decadeonrestoration.org/what-decade).

Many studies relate high biodiversity and high endangered plant persistence in species-rich grasslands to phosphorus or nitrogen (co)-limitation (Olde Venterink 2011; Ceulemans et al. 2014; Wassen et al. 2020). Phosphorus removal appears of the utmost importance since high soil phosphorus concentrations are detrimental for restoring species-rich grasslands (Fagan et al. 2008; Ceulemans et al. 2011; Merunkova & Chytry 2012). Many European agricultural fields and grasslands have high stocks of soil phosphorus due to past fertilization adding more phosphorus than the crops could use (Sattari et al. 2012). Phosphorus accumulated in the soil often during decades, and due to its immobile nature (Stevenson & Cole 1999), multiple decades or more may be needed to restore phosphorus-poor soil conditions (Dupouey et al. 2002; McLauchlan 2006). To restore species-rich grasslands, practitioners, therefore, attempt to lower the phosphorus availability in the soil by mowing and removing nutrients within the cut hay. After abiotic restoration, time and effort for biotic restoration is often required due to the lack of nearby relict populations or a viable seed bank (Bakker & Berendse 1999; Kiehl et al. 2010).

Here we examine the feasibility of restoring species-rich grasslands on former agricultural land in Flanders (northern Belgium). For 507 grasslands mainly situated in 12 different Natura 2000 SACs, we estimated the suitability for abiotic restoration, focussing on removal of excess soil phosphorus. We estimated the necessary duration of phosphorus removal to show whether abiotic restoration is realistic before 2030 or 2050. We considered two phosphorus removal methods, traditional mowing with the removal of hay and P-mining, in order to aid decision makers management choices. While depleting phosphorus from the soil by mowing can take a very long time due to other elements becoming limiting (Oelman et al. 2009), P-removal is accelerated by P-mining through nitrogen and potassium fertilization (Crawley et al. 2005). Our time estimation involves selecting a bioavailable soil P-target and simulating the restoration time required to reach the P-target with the chosen restoration technique (Schelfhout et al. 2017). We also modelled the relation between bioavailable and slowly cycling phosphorus pool can feed the bioavailable phosphorus pool for many years (Johnston et al. 2014). This was taken into account in our time estimation. In our calculations, we only focussed on abiotic restoration and did not include the extra time needed for biotic restoration.

Methods

Dataset used for simulations

During the period 2012-2018, a total of 507 grasslands mainly spread over 12 SACs in Flanders (northern Belgium) were studied in the framework of ecological restoration projects aiming to restore different Natura 2000 grassland types (H2130, H2150, H2190, H6130, H6210, H6230*, H6410 & H6510; see Table S1). The historical land-use intensity of these grasslands ranged from probably no fertilization (extensive grassland management) to large amounts of fertilization (intensive grassland or even arable management). Grasslands were spread over different mineral soil textures and drainage gradients. We sampled the soil at two to eight sampling points per grassland depending on its size. We avoided the field edges by at least 10 m and sampling points were spread equally over the whole grassland. In each sampling point, the soil was sampled using a three centimetre wide auger on several depths between 0 and 50 cm (see Table S1). For each grassland, point samples were mixed per soil depth (zone sampling) in order to obtain average field values and to reduce chances of selecting a single nonrepresentative soil sample. As an exception, the fields from one study site ('Smeetshof', see Table S1) were sampled using grid sampling: first, a grid was placed over the entire study site, then, each grid point was sampled (2 to 3 points per field) and samples were analysed separately. Finally, these measured grid point values were converted to field level (see Table S1) in order to make all study sites comparable. For data cleaning and chemical analyses, see Supplement S1.

P-targets for abiotic restoration

Most species-rich grassland types require oligotrophic or mesotrophic soil conditions, more specifically, phosphorus-poor soils to sustain a diverse plant community (Gowing et al. 2002; Gilbert et al. 2009, Wassen et al. 2020). In this context, phosphorus-poor can be defined by low bioavailable P-concentrations (bioavailable or Olsen phosphorus, P_{ols}), namely a maximum of 10 mg bioavailable P_{ols} kg⁻¹ dry soil for oligotrophic grasslands as Nardus grasslands (H6230*) and 15 mg bioavailable P_{ols} kg⁻¹ dry soil for mesotrophic grasslands as Lowland Hay meadows (H6510), based on T'jollyn et al. 2009). Bioavailable P combines phosphorus in the soil solution and soil surface-adsorbed phosphorus. This pool correlates well with plant-uptake of phosphorus within one growing season in species-rich grasslands (Gilbert et al. 2009).

Bioavailable phosphorus concentrations greater than these targets will probably not suffice to reach the EU goals because (threatened) key species may not be able to establish and thrive (Wassen et al. 2020) since competition for light might be too high (Hautier et al. 2009).

Simulation of the time required to reach the P-targets

Following the decision-making scheme in Schelfhout et al. (2017), we first assessed which grasslands did not need abiotic restoration, *i.e.* contained less bioavailable P in the top 20 cm soil layer than the P-targets. These grasslands were counted and excluded from the time calculations for abiotic restoration. These grasslands however, do need biotic restoration, but this assessment was out of the scope of this study.

For each grassland that contained more bioavailable P in the top 20 cm soil layer than the P-targets, we simulated the time required to reach the P-targets. Therefore, we needed to consider two P-pools (Schelfhout et al. 2019): the bioavailable P-pool and the slowly cycling P-pool. Slowly cycling phosphorus consists of less readily available and extractable phosphorus (P_{ox}) which is bound with calcium (high soil pH) or with iron and aluminum (low soil pH; Lijklema 1980; Stevenson & Cole 1999). This phosphorus pool can become available for plant-uptake in the long-term (van Rotterdam et al. 2012) because it is in equilibrium with the bioavailable phosphorus pool. When the bioavailable phosphorus pool diminishes due to plant-uptake, it becomes replenished from the slowly cycling phosphorus pool (Johnston et al. 2014).

The time required to reach the bioavailable P-targets was performed by modelling 'annual loops' where the excessive amount of soil phosphorus was decreased with the amount of phosphorus produced with aboveground biomass, i.e. phytomining soil phosphorus (see Fig. 1 for a schematic overview and Chapter 7 in Schelfhout 2019). First, we calculated the distance to the P-target by comparing the initial (at year n) and target bioavailable phosphorus concentrations. If the initial bioavailable phosphorus concentration was greater than the P-target, we estimated how much phosphorus is removed with biomass at year n with the selected technique, depending on the bioavailable phosphorus concentration at year n and the soil type. Then, we calculated the remaining slowly cycling phosphorus stock at year n+1. Finally, we calculated the bioavailable phosphorus concentration to the P-target, we stopped the loop and counted the number of loops representing the number of years required for reaching the P-target by the selected restoration technique. Then we examined what percentage of the grasslands will be able to reach the P-targets before the UN-target of 2030 and the EU-target of 2050.

Annual P-removal with biomass

In each simulation loop, we estimated annual phosphorus removal with biomass with two restoration techniques of phytomining. The first technique, 'mowing', is the practice of cutting grassland swards two or three times a year to remove nutrients with hay and without using fertilizers. After several years of mowing without fertilization, biomass production decreases significantly due to limitation of nutrients other than phosphorus, namely nitrogen (Van Der Woude et al. 1994) and potassium (Oelmann et al. 2009). Due to low biomass production (3 ton dry matter $ha^{-1} \cdot y^{-1}$), phosphorus removal is generally also low (8 kg P $ha^{-1} \cdot y^{-1}$ on sandy soils; Schelfhout et al. 2019).

The second technique, 'P-mining', combines the mowing technique with fertilization of nitrogen and potassium to maximize biomass production and thus phosphorus removal. Biomass production and phosphorus removal by P-mining are generally higher than by mowing (7 ton dry matter ha⁻¹ y⁻¹ and 18 kg P ha⁻¹ y⁻¹ on sandy soils; Schelfhout et al. 2019). Because the potential to remove phosphorus with biomass depends on the current concentration of bioavailable phosphorus in the soil (Johnston et al. 2016), the difference between the two restoration techniques diminishes with declining bioavailable phosphorus (Schelfhout et al. 2019). Below 25 mg P_{ols} kg⁻¹, the advantage of P-mining over mowing appears to be small. Therefore, in our calculations we assumed P-mining management was switched to mowing management when the soil contained less than 25 mg P_{ols} kg⁻¹.

Phosphorus removal with biomass also depends on the soil type (Johnston et al. 2016). Hence, for sandy soils, we assumed the annual phosphorus removal rates as described by the linear regressions from Schelfhout et al. (2019):

$$P_{removal through mowing} = 4.8 (\pm 3.2) + 0.05 (\pm 0.03) \cdot P_{ols}$$
(Equation 1)
$$P_{removal through P-mining} = 9.8 (\pm 5.5) + 0.11 (\pm 0.07) \cdot P_{ols}$$
(Equation 2)

For more loamy or clayey soil types linear regressions were made (function *lm*, *stats* package (R core Team 2020)) with the mowing and P-mining data from the HerBioGras project of the University of Applied Sciences and Arts (Vanhellemont M. et al. unpublished data):

$$P_{removal\ through\ mowing} = 2.4\ (\pm 1.9) + 0.23\ (\pm 0.02) \cdot P_{ols}$$
 (Equation 3)

$$P_{removal through P-mining} = 22.0 (\pm 3.0) + 0.22 (\pm 0.04) \cdot P_{ols}$$
(Equation 4)

To account for the annual variation in P-removal in equations 1-4, we calculated 1000 bootstrap replicates for each of the coefficients using their average and standard deviation (See Supplement S2). Normality was tested using the Shapiro-Wilk test (*stats* package (R core Team 2020)), homoscedasticity

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with the studentized Breusch-Pagan test from the *Imtest* package in R (Zeileis & Hothorn 2002); kurtosis and skewness were tested using the *e1071* package in R (Meyer et al. 2019).

Remaining slowly cycling phosphorus at year n+1

The slowly cycling phosphorus concentration at year n (in mg P kg⁻¹) was converted to the slowly cycling phosphorus stock at year n (in kg P ha⁻¹) by assuming a rooting depth of 20 cm (valid for most grassland species according to Jumpponen et al. 2002 and De Boer et al. 2020) and an average soil bulk density of 1.3 kg·dm⁻³ (Vanhoof *et al.* 2010). In each simulation loop, we subtracted the slowly cycling phosphorus stock with the estimated amount of phosphorus removed with biomass to simulate the annual depletion of phosphorus. The remaining slowly cycling phosphorus stock at year n+1 was again converted to concentration under the same assumptions. When moving through the simulation loops, we assumed that other soil traits than phosphorus concentrations (*e.g.* iron, ...) remained constant.

Remaining bioavailable phosphorus at year n+1

In each simulation loop, we predicted the remaining bioavailable phosphorus concentration at year n+1 from the remaining slowly cycling phosphorus concentration at year n+1. Since the relation between bioavailable and slowly cycling phosphorus varies across soil types (Johnston et al. 2016) and the soil data in this study contains multiple soil types (see Table S1), we looked for a 'universal' fit between bioavailable and slowly cycling phosphorus in our dataset (for the complete method see Supplement S2). In brief, we made two linear models: a minimal model, obtained by backward model selection from a full model and a basic model, composed by the two most important explanatory input variables, namely the slowly cycling phosphorus concentration and the total iron concentration, and their interaction term. We chose to continue our simulation loop with the basic model only, due to the great comparability between the basic and the minimal model (R² = 76% and 82% respectively; see Table S2). Besides this, the basic model can be used for a larger number of grasslands since not all variables integrated in our minimal model were measured for all grasslands. A less comprehensive soil analysis is also more feasible for managers, making the basic model an easy-to-use tool in practice.

Simply equating P_{ols} at year n+1 to the theoretical P_{ols} estimated by our basic model would result in large error margins since this model is based on all data points. It thus represents an "average relation" between P_{ols} and P_{ox} , and the following method to calculate P_{ols} at year n+1 was chosen in order to take into account that some data points have a rather high or low P_{ols}/P_{ox} ratio:

$$P_{ols} \text{ at year } (n+1) = \frac{\text{estimated } P_{ols} \text{ at year } (n+1)}{\text{estimated } P_{ols} \text{ at year } n} \cdot P_{ols} \text{ at year } n$$

Since a rooting depth of 20 cm is assumed, only soil samples that were (partly) taken within the first 20 cm of soil were used in this loop. The final mowing and P-mining times for a certain field were then calculated by adding up the mowing and P-mining times of these soil samples. For soil samples that were only partly taken within the first 20 cm of soil, an equally large part of their mowing and P-mining times were added.

The data was analysed in R, version 4.0.0 (R Core Team 2020) and plots were made using the *ggplot*-function from the *ggplot2* r-package (Wickham 2016). For the calculation of the 95% confidence intervals and the assessment of the importance of other soil parameters, see Supplement S2.

Results

The 507 grasslands in our database contained a broad range of P-concentrations, Fe-concentrations, soil acidity, and soil textures (Tables 1 and S1). The bioavailable P-concentration was correlated significantly with the slowly cycling P-concentration (see Fig. S1), however we found poorer correlations between bioavailable P and the other soil parameters (see Table S3). The total Fe-concentration was significantly inversely related to the P_{ols}/P_{ox} ratio (linear regression with the inverse of the square root of iron as predictor variable, all *P*-values < 0.001, R² = 0.32, Fig. 2). Non-sandy soils generally contain higher Fe-concentrations than sandy soils (all *P*-values < 0.001, R² = 0.28, Fig. 2).

With the basic model as regression, we calculated the proportion of grasslands that have reached the P-targets of 10 mg P_{ols} kg⁻¹ (oligotrophic grassland types) and 15 mg P_{ols} kg⁻¹ (mesotrophic grassland types, between brackets in the text) after 0, 10 and 30 years of mowing or P-mining management (Fig. 3). Only 11% (18%) of all grasslands currently meet the P-targets. After 10 years, $6 \pm 1\%$ ($12 \pm 4\%$) of all other grasslands can meet these targets by mowing. P-mining does not significantly restore a higher proportion of grasslands ($8 \pm 3\%$ ($18 \pm 8\%$), Welch's t-tests, P > 0.05). After 30 years of P-removal, the proportion of grasslands can be raised further until 24 ± 3% ($35 \pm 4\%$) by mowing and $35 \pm 10\%$ ($68 \pm 9\%$) by P-mining. Within 30 years, P-mining has a significant effect on the proportion of grasslands (32.5%) the bioavailable P-concentration was already below 25 mg P_{ols} kg⁻¹, *i.e.* the bioavailable P-concentration when P-removal by P-mining is likely no longer higher than by mowing.

The required time to reach the P-targets by mowing and P-mining increased with the total iron concentration (Figs 4a-b, all *P*-values < 0.001, adj. $R^2 = 0.40$ for mowing and adj. $R^2 = 0.24$ for P-mining) and decreased with the ratio of bioavailable to slowly cycling phosphorus (Figs 4c-d, all *P*-values < 0.001, adj. $R^2 = 0.41$ for mowing and adj. $R^2 = 0.26$ for P-mining).

Discussion

Soil phosphorus puts a mortgage on abiotic restoration

We evaluated the abiotic restoration potential, with a focus on removal of excess soil phosphorus, for 507 grasslands, all destined for species-rich grassland restoration. The historical land-use intensity of these grasslands ranged from probably no fertilization (extensive grassland management) to large amounts of fertilization (intensive grassland or arable management). We chose a maximum time limit of 10 and 30 years in agreement with the UN and EU objectives to restore different types of species-rich grassland habitats by 2030 and 2050, respectively. Most of the species-rich grassland types need bioavailable P-concentrations less than 10 or 15 mg P_{ols} kg⁻¹ for optimal potential (Gowing et al. 2002; Gilbert et al. 2009). Hence, we chose these two P-targets for oligotrophic grasslands as Nardus grasslands (H6230*) and mesotrophic grasslands as Lowland Hay meadows (H6510), respectively.

Only 11% (18.0%) of all studied grasslands were found to currently fulfil these abiotic targets. This low proportion of oligotrophic and mesotrophic grasslands was expected because Flanders is a region typified with high livestock production and consequently large phosphorus surpluses (De Smet et al. 1996; Bomans et al. 2005). These P-poor grasslands probably never received (high amounts of) fertilizers or manure and should receive high-priority conservation (cf. Moore et al. 1989 for oligotrophic wetlands) because restoration of these P-poor soil conditions on eutrophic soils is nearly impossible without drastic and expensive measures such as topsoil removal. It is likely, however, that these grasslands are species-poor due to habitat fragmentation (Schneiders et al. 2020) and require biotic restoration (similar to Schelfhout et al. 2017; see further in this discussion).

Additionally to the P-poor grasslands, 6 to 12% of the grasslands may reach the oligotrophic and mesotrophic P-targets by 2030. By 2050 this proportion increases to a quarter and a third. P-mining does not increase this proportion significantly within 10 years, since most grasslands that can reach the P-target within this time limit already have bioavailable P-concentrations below 25 mg P_{ols} kg⁻¹. However, within 30 years P-mining does significantly raise this proportion to a third of the grasslands for the oligotrophic target and two thirds for the mesotrophic target. The calculated percentages of

grasslands reaching these P-targets within these time limits should, however, for several reasons discussed below, be seen as an optimistic scenario and potentially an overestimation.

Phosphorus removal by mowing and P-mining depends on the daily practice of managers: the exact time of year when mowing and the amount of cuts per year will affect phosphorus removal efficiency. Besides this, more frequent extreme drought periods during future summers may reduce net primary production and thus decrease yearly phosphorus removal (Sala et al. 1988; Zhao & Running 2010).

Further, abiotic restoration may also require other changes to the environment such as lowering atmospheric nitrogen deposition (Bobbink et al. 2010; Stevens et al. 2011) and/or increasing groundwater levels (Oomes & Van der Werf 1996; Schrautzer et al. 1996). We did not account for the time needed for these changes. Next to time for abiotic restoration, restoration also requires time for the biotic recovery of the grassland ecosystem (Schelfhout et al. 2017). After years or decades of agricultural use, most typical grassland species disappeared and species recolonisation can be delayed by habitat fragmentation, the lack of nearby relict populations and the lack of viable seed banks (Huxel & Hastings 1999; Bakker & Berendse 1999). Assisted reintroductions will often be necessary (Maunder 1992; Hodder & Bullock 1997; Godefroid et al. 2011). Especially for grasslands where P-mining is practiced biotic restoration is postponed due to the addition of nitrogen and potassium and the potential cultivation of crops (Schelfhout et al. 2017), whereas biotic and abiotic restoration occur simultaneously when mowing management is used as a restoration technique. A benefit of P-mining, however, is that it can ensure cooperation with farmers since the production of high quality hay due to higher nitrogen --and thus protein- levels is guaranteed, an important measure of feed quality of hay for dairy cows (Satter & Roffler 1975). The sale hereof may balance costs and even some small profit might be made.

For all grasslands that will not reach the P-target within the desired time frame by mowing or P-mining, topsoil removal could be considered (when permitted by, among others, hydrological conditions). At present, topsoil removal is often not feasible because of the high costs (Klimkowska et al. 2010) - although the cumulative cost of yearly mowing can sometimes surpass the one-time cost of topsoil removal (Smolders et al. 2008). Multiple studies, though, have shown that the combination of topsoil removal and the transfer of hay from intact species-rich grasslands can result in successful and fast establishment of target vegetations (e.g. Patzelt et al. 2001; Hölzel & Otte 2003; Klimkowska et al. 2009). In addition, topsoil removal might be of particular interest for fields where the restoration of high groundwater levels is essential (Rasran et al. 2007).

When topsoil removal proves to be impossible, different less phosphorus critical grassland types can be pursued. These grasslands can still be restored towards flower-rich grasslands (probably without key species), supporting pollinators and possibly creating a buffer zone around more valuable fields (Wratten et al. 2012; Woodcock et al. 2014).

The mortgage imposed by elevated soil phosphorus concentrations on grassland restoration becomes clear by our evaluation of the current abiotic potential and modelling of the opportunities for ecological restoration. Overcoming the extinction debt, in this case the ongoing loss of specialist grassland species in a nutrient-enriched landscape, calls for targeted habitat restoration (Kuussaari et al. 2009), a step-by-step strategy. As phosphorus-poor fields are rare, protection of these specific soil conditions and biotic restoration on these fields should be prioritized as these can be or become 'diversity hotspots' (cfr. Cousins & Eriksson 2008) where specialist species on the verge of disappearance can find refuge. This action would 'buy' us more time as other fields where abiotic restoration is feasible within a reasonable timeframe are being restored and become suitable for these species. The landscape scale should also be considered in the selection of where to aim grassland restoration efforts; preferably the hotspots become connected (Cousins & Eriksson 2008). Therefore, large investments will have to be done both in restoration efforts and in the search for fields suitable for restoration. The UN call for a decade of ecosystem restoration may speed up these actions. To our knowledge, no similar predictive models have been made for other parts of Europe, although many other countries than Belgium have similar intensive agricultural histories with high historical fertilization rates (Green 1990; Muller et al. 1998; Willems 2001). Therefore, we argue that many other European countries might be facing similar restoration obstacles to reach the EU objectives before 2050.

Iron concentrations matter

Besides the soil P-stocks, the iron concentrations appear to influence the required mowing or P-mining time. As a soil contains more iron, a larger fraction of phosphorus can be withheld in the slowly cycling P-pool. Iron-rich soils, therefore, generally have lower P_{ols}/P_{ox} -ratios. Only a small fraction of the phosphorus stock will be removed yearly by mowing or P-mining, while the small bioavailable P-stock will be replenished from the large slowly cycling phosphorus stock. In iron-poor soils with generally high P_{ols}/P_{ox} ratios, large proportions of the phosphate stock can be removed in short time spans. Consequently, soils with similar P_{ols} -concentrations might take very different time spans to deplete depending on their iron content. Generally, soils with low sand fractions tend to have high Fe_{tot} -concentrations, and thus low P_{ols}/P_{ox} -ratios. Although sandy soils are less productive than other soil textures (compare the P-removal potential in Equations 1-2 versus Equations 3-4), P_{ols} -stocks in these soils are likely depleted faster than for other soil textures.

Further improvement of our predictions could be accomplished by incorporating models describing the relation between P_{ols} -concentration and yearly phosphorus removal for more distinct soil textures and drainage classes. Furthermore the importance of aluminum and calcium could be assessed. Aluminum is, just as iron, known to bind phosphate at low pH values (Lijklema 1980), while calcium binds phosphates at higher pH values (Weng et al. 2011) and might thus also lower the P_{ols}/P_{ox} ratio.

As a soil bulk density of 1.3 kg·dm⁻³ was assumed for all soils, predictions might over- or underestimate required time spans respectively for lighter and heavier soils. Besides this, a rooting depth of 20 cm was assumed (Jumpponen et al. 2002; De Boer et al. 2020). Since rooting depth might alter according to soil bulk density (Lampurlanés & Cantero-Martínez 2003) and water availability (Carrow 1996) mowing and P-mining times can slightly deviate.

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References

Bakker J, Berendse F (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology & Evolution 14(2):63–68

Bobbink R, Hicks K, Galloway J, Spranger T, Alkemade R, Ashmore M, Bustamante M, Cinderby S, Davidson E, Dentener F, Emmett B, Erisman JW, Fenn M, Gilliam F, Nordin A, Pardo L, De Vries W (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20:30–59

Bomans E, Fransen K, Gobin A, Mertens J, Michiels P, Vandendriessche H, Vogels N (2005) Addressing phosphorus related problems in farm practice. Commissioned by the European Commission

Carrow RN (1996). Drought Resistance Aspects of Turfgrasses in the Southeast: Root-Shoot Responses. Crop Science 36(3):687

CBD/COP/14/9 2018. Long-term strategic directions to the 2050 vision for biodiversity, approaches to living in harmony with nature and preparation for the post-2020 global biodiversity framework. Fourteenth meeting, agenda item 17

Ceulemans T, Merckx R, Hens M, Honnay O (2011) A trait-based analysis of the role of phosphorus vs. nitrogen enrichment in plant species loss across North-west European grasslands. Journal of Applied Ecology 48:1155–1163

Ceulemans T, Stevens CJ, Duchateau L, Jacquemyn H, Gowing DJG, Merckx R, Wallace H, van Rooijen N, Goethem T, Bobbink R, Dorland E, Gaudnik C, Alard D, Corcket E, Muller S, Dise NB, Dupré C, Diekmann M, Honnay O (2014) Soil phosphorus constrains biodiversity across European grasslands. Global Change Biology 20: 3814–3822

COP Decision X/2 2010. Decision adopted by the conference of the parties to the convention on biological diversity at its tenth meeting X/2. The Strategic Plan for Biodiversity 2011-2020 and the Aichi Biodiversity Targets. Tenth meeting, agenda item 4.4

Cousins SAO, Eriksson O (2008) After the hotspots are gone: Land use history and grassland plant species diversity in a strongly transformed agricultural landscape. Applied Vegetation Science 11(3): 365–374

Crawley MJ, Johnston AE, Silvertown J, Dodd M, de Mazancourt C, Heard MS, Henman DF, Edwards GR (2005) Determinants of species richness in the Park Grass Experiment. The American Naturalist 165: 179–192

Dallal GE (2001) Collinearity. http://www.tufts.edu/~gdallal/collin.htm.

Decision No 1386/2013/EU of the European Parliament and of the Council of 20 November 2013 on a General Union Environment Action Programme to 2020 'Living well, within the limits of our planet' Text with EEA relevance

Dengler J, Janišovác M, Török P, Wellstein C (2014) Biodiversity of Palaearctic grasslands: a synthesis. Agriculture, Ecosystems & Environment 182:1–14

De Boer HC, Deru JGC, Van Eekeren N (2020) Sward lifting in compacted grassland: contrasting effects on two different soils. Soil & Tillage Research 201 <u>https://doi.org/10.1016/j.still.2019.104564</u>

De Smet J, Hofman G, Vanderdeelen J, Van Meirvenne M, Baert L (1996) Phosphate enrichment in the sandy loam soils of West-Flanders, Belgium. Fertilizer Research 43:209–215

Dupouey JL, Dambrine E, Laffite JD, Moares C (2002) Irreversible impact of past land use on forest soils and biodiversity. Ecology 83:2978-2984

Habitats Directive 92/43/EEC 1992. The conservation of natural habitats and of wild fauna and flora

Fagan KC, Pywell RF, Bullock JM, Marrs RH (2008) Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. Journal of Applied Ecology 45:1293-1303.

Gilbert JC, Gowing DJG, Wallace H (2009) Available soil phosphorus in semi-natural grasslands: Assessment methods and community tolerances. Biological Conservation 142(5):1074-1083.

Godefroid S, Piazza C, Rossi G, Buord S, Stevens A, Aguraiuja R, Cowell W, Weekley CW, Vogg G, Iriondo JM, Johnson I, Dixon B, Gordon D, Magnanon S, Valentin B, Bjureke K, Koopman R, Vicens M, Vanderborght T (2011) How successful are plant species reintroductions? Biological Conservation 144:672-682

Gowing DJG, Tallowin JRB, Dise NB, Goodyear J, Dodd ME, Lodge RJ (2002) A review of the ecology, hydrology and nutrient dynamics of floodplain meadows in England. Peterborough: Natural England 446:85

Green BH (1990) Agricultural intensification and the loss of habitat, species and amenity in British grasslands: a review of historical change and assessment of future prospects+. Grass and Forage Science 45(4):365–372

Green PA, Vörösmarty CJ, Meybeck M, Galloway JN, Peterson BJ, Boyer EW (2004) Pre-industrial and contemporary fluxes of nitrogen through rivers: a global assessment based on typology. Biogeochemistry 68:71-105

Grime JP (2001) Plant strategies, vegetation processes and ecosystem properties. John Wiley & Sons, Chichester, UK

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

Hejcman M, Hejcmanová P, Pavlů V, Beneš J (2013) Origin and history of grasslands in Central Europe – a review. Grass and Forage Science 68:345–363 Hodder K, Bullock JM (1997) Translocations of native species in the UK: implications for biodiversity. Journal of Applied Ecology 34:547-565

Hölzel N, Otte A (2003) Restoration of a species-rich flood meadow by topsoil removal and diaspore transfer with plant material. Applied Vegetation Science 6:131–140

Huxel GR, Hastings A (1999) Habitat Loss, Fragmentation, and Restoration. Restoration Ecology 7(3):309–315

Johnston AE, Poulton PR, Fixen PE, Curtin D (2014) Phosphorus. Its Efficient Use in Agriculture., 1st ed. Advances in Agronomy 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 and Management 32(2)151-161

Jumpponen A, Högberg P, Huss-Danell K, Mulder CPH (2002) Interspecific and spatial differences in nitrogen uptake in monocultures and two-species mixtures in north European grasslands. Functional ecology 16:454-461

Kiehl K, Kirmer A, Donath TW, Rasran L, Hölzel N (2010) Species introduction in restoration projects – Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. Basic and Applied Ecology 11(4):285–299

Klimkowska A, Kotowski W, Van Diggelen R, Grootjans AP, Dzierża P, Brzezińska K (2009) Vegetation re-development after fen meadow restoration by topsoil removal and hay transfer. Restoration Ecology 18(6):924–933

Klimkowska A, Dzierza P, Brzezinska K, Kotowski W, Medrzycki P (2010) Can we balance the high costs of nature restoration with the method of topsoil removal? Case study from Poland. Journal for Nature Conservation 18:202–205

Kuussaari M, Bommarco R, Heikkinen RK, Helm A, Krauss J, Lindborg R, Öckinger E, Pärtel M, Pino J, Rodà F, Stefanescu C, Teder T, Zobel M, Steffan-Dewenter, I (2009) Extinction debt: a challenge for biodiversity conservation. Trends in Ecology and Evolution 24(10): 564–571 https://doi.org/10.1016/j.tree.2009.04.011

Lampurlanés J, Cantero-Martínez C (2003) Soil Bulk Density and Penetration Resistance under Different Tillage and Crop Management Systems and Their Relationship with Barley Root Growth. Agronomy Journal 95:525–536

Lijklema L (1980) Interaction of orthophosphate with iron(III) and aluminum hydroxides. Environmental Science & Technology 14(5):537–541

Maunder M (1992) Plant reintroduction: an overview. Biodiversity & Conservation 1:51-61

McLauchlan K (2006) The nature and longevity of agricultural impacts on soil carbon and nutrients: a review. Ecosystems 9:1364–1382

Merunkova K, Chytry M (2012) Environmental control of species richness and composition in upland grasslands of the southern Czech Republic. Plant Ecology 213:591–602

Meyer D, Dimitriadou E, Hornik K, Weingessel A, Leisch F (2019) e1071: Misc Functions of the Departement of Statistics, Probability Theory Group (Formerly: E1071), TU Wien. R package version 1.7-2. URL https://cran.reg/package=e1071

Moore DRJ, Keddy PA, Gaudet CL, Wisheu IC (1989) Conservation of wetlands: Do infertile wetlands deserve a higher priority? Biological Conservation 47:203–217. doi: 10.1016/0006-3207(89)90065-7

Muller S, Dutoit T, Alard D, Grevilliot F (1998) Restoration and Rehabilitation of Species-Rich Grassland Ecosystems in France: a Review. Restoration Ecology 6(1):94–101

Oelmann Y, Broll G, Holzel N, Kleinebecker T, Vogel A, Schwartze P (2009) Nutrient impoverishment and limitation of productivity after 20 years of conservation management in wet grasslands of northwestern Germany. Biological Conservation 142:2941–2948

Olde Venterink H (2011) Does phosphorus limitation promote species-rich plant communities? Plant and Soil 345:1–9

Oomes MJM & Van der Werf A (1996) Restoration of species diversity in grasslands: The effect of grassland management and changes in ground water level. Acta Botanica Gallica 143(4-5):451–461

Patzelt A, Wild U, Pfadenhauer J (2001) Restoration of wet fen meadows by topsoil removal: vegetation development and germination biology of fen species. Restoration Ecology 9(2):127–136

Poschlod P, Baumann A, Karlik P (2009) Origin and development of grasslands in Central Europe. In: Veen P, Jefferson R, de Smidt J, van der Straaten J (Eds.), Grasslands in Europe of High Nature Value. KNNV Publishing, Zeist 15–26

R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Rasran L, Vogt K, Jensen K (2007) Effects of topsoil removal, seed transfer with plant material and moderate grazing on restoration of riparian fen grasslands. Applied Vegetation Science 10:451-460

Sala OE, Parton WJ, Joyce LA, Lauenroth WK (1988) Primary production of the central grassland region of the United States. Ecology 69(1):40-45

Sattari SZ, Bouwman AF, Giller KE, Van Ittersum MK (2012) Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. PNAS 109(16):6348-6353

Satter LD, Roffler RE (1975) Nitrogen requirement and utilization in dairy cattle. Journal of Dairy Science 58(8):1219–1237

Schelfhout S, Mertens J, Perring MP, Raman M, Baeten L, Demey A, De Schrijver A (2017) P-removal for restoration of Nardus grasslands on former agricultural land: cutting traditions. Restoration Ecology 25:178–187

Schelfhout S (2019) Restoration of species-rich Nardus grasslands via phosphorus-mining. Doctoral dissertation, UGent, Gent, Belgium. Retrieved from https://biblio.ugent.be/

Schelfhout S, De Schrijver A, Vanhellemont M, Vangansbeke P, Wasof S, Perring MP, Haesaert G, Verheyen K, Mertens J (2019) P-mining to re-establish phosphorus-poor soil conditions for nature restoration on former agricultural land. Plant and soil 440(1-2):233-246

Schneiders A, Alaerts K, Michels H, Stevens M, Van Gossum P, Van Reeth W, Vught I (2020) Natuurrapport 2020. Instituut Natuur- en Bosonderzoek – INBO

Schrautzer J, Asshoff M, Müller F (1996) Restoration strategies for wet grasslands in Northern Germany. Ecological Engineering 7(4):255–278

Smolders AJP, Lucassen ECHET, van der Aalst M, Lamers LPM, Roelofs JGM (2008) Decreasing the abundance of *Juncus effusus* on former agricultural lands with noncalcareous sandy soils: possible effects of liming and soil removal. Restoration Ecology 16:240–248

Stevens CJ, Gowing DJG, Wotherspoon KA, Alard D, Aarrestad PA, Bleeker A, Bobbink R, Diekmann M, Dise NB, Duprè C, Dorland E, Gaudnik C, Rotthier S, Soons MB, Corcket E (2011) Addressing the Impact of Atmospheric Nitrogen Deposition on Western European Grasslands. Environmental Management 48(5):885–894

Stevenson FJ, Cole MA (1999) Cycles of Soil (Carbon, Nitrogen Phosphorus Sulfur, Micronutrients). John Wiley and Sons Publishers, Hoboken, pp. 279–329, The Phosphorus Cycle

T'Jollyn F, Bosch H, Demolder H, De Saeger S, Leyssen A, Thomaes A, Wouters J, Paelinckx D, Hoffmann M (2009) Ontwikkeling van criteria voor de beoordeling van de lokale staat van instandhouding van de Natura 2000 habitatypen. Versie 2.0. Instituut voor natuur- en bosonderzoek, Brussel.

Van Der Woude BJ, Pegtel DM, Bakker JP (1994) Nutrient limitation after long-term nitrogen fertilizer application in cut grasslands. Journal of Applied Ecology 31:405–412

Vanhoof C, De Wit J, Wouters W, Tirez K (2010) Houdbaarheid van bodemmonsters voor de bepaling van nitraatresidu. Study commissioned by the Vlaamse Landmaatschappij (Flemish Land company)

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

Wassen MJ, Schrader J, van Dijk J, Eppinga MB (2020) Phosphorus fertilization is eradicating the niche of northern Eurasia's threatened plant species. Nature Ecology & Evolution 5:67 - 73

Weng L, Vega FA, Van Riemsdijk WH (2011) Competitive and Synergistic Effects in pH Dependent Phosphate Adsorption in Soils: LCD Modeling. Environmental Science & Technology 45(19):8420– 8428

Wickham H (2016) 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. Journal of Vegetation Science 23:796-802 doi: 10.1111/j.1654-1103.2012.01400.x

Willems JH (2001) Problems, Approaches, and Results in Restoration of Dutch Calcareous Grassland During the Last 30 Years. Restoration Ecology 9(2):147–154

Woodcock BA, Savage J, Bullock JM, Nowakoski M, Orr R, Tallowin JRB, Pywell RF (2014) Enhancing floral resources for pollinators in productive agricultural grasslands. Biological Conservation 171:44-51 doi: 10.1016/j.biocon.2014.01.023

Wratten SD, Gillespie M, Decourtye A, Mader E, Desneux N (2012) Pollinator habitat enhancement : Benefits to other ecosystem services. Agriculture, ecosystems & environment 159:112-122 doi: 10.1016/j.agee.2012.06.020

Zeileis A, Hothorn T (2002) Diagnostic checking in Regression Relationships. R News 2(3):7-10 https://CRAN/R-project.org/doc/Rnews/ Zhao M, Running SW (2010) Drought-induced reduction in global net primary production from 2000 through 2009. Science 329(5994):940-943

Figures and tables

Table 1

Table 1: Average, standard deviation and range of the chemical soil properties, measured within the top layer (0 - 20 cm) of the 507 grasslands

Variable	Average ± standard deviation	Minimum - maximum	Number of grasslands where chemical property was measured
Pols	41.5 ± 28.2 mg/kg	< 1.0* – 174.1 mg/kg	507
Pox	331.2 ± 273.0 mg/kg	9.8 – 2693.6 mg/kg	507
Ptot	792.0 ± 426.1 mg/kg	139.7 – 4029.1 mg/kg	506
Fetot	16.0 ± 10.3 g/kg	1.8 – 60.7 g/kg	507
Catot	8.0 ± 16.2 g/kg	0.0118 – 146.4 g/kg	404
рН _{Н20}	6.31 ± 0.82	4.29 - 8.85	488
C _{tot}	3.72 ± 2.27 %	0.48 - 17.04 %	365
Stot	0.055 ± 0.036 %	0.009 - 0.356 %	365

* P_{ols} -concentration was below the detection limit of 1.0 mg P_{ols} kg⁻¹

Figure 1



Fig. 1: Schematic overview of an annual loop. Step 1 corresponds to the phosphorus removal in function of the bioavailable phosphorus. Step 2 calculates the remaining slowly cycling phosphorus. Step 3 and 4 represent the chemical equilibrium between the slowly cycling phosphorus and the bioavailable phosphorus through which the concentration of the latter can be calculated for the next year.



Figure 2



Fig. 2: P_{ols}/P_{ox} ratio (no unit) in function of the square root of iron concentration (mg Fe_{tot} kg⁻¹ dry soil). Soils with a low sand fraction are clearly more Fe-rich, and thus had an overall lower P_{ols}/P_{ox} ratio. Data points where P_{ols} -concentration was below the detection limit (48 points) were removed as for data points with a P_{ols}/P_{ox} -ratio over 1.







Fig. 3: The proportion of grasslands (n=452 for 10 mg P_{ols} kg⁻¹ and 416 for 15 mg P_{ols} kg⁻¹) that have reached the two P-targets (blue: 10 mg P_{ols} kg⁻¹; green: 15 mg P_{ols} kg⁻¹) after 10 and 30 years with P-removal by mowing (light colors) or with P-removal by P-mining (full colors). 95% confidence intervals are indicated for our calculated results by flags. Significant differences between mowing and P-mining are indicated with asterisks. Significance levels: n.s. P > 0.05; * 0.01 < $P \le 0.05$; ** 0.001 < $P \le 0.01$; *** P < 0.001.



Figure 4



Fig. 4: Predicted mowing time (4a and c, years) and P-mining time (4b and d, years) in function of P_{ols} -concentration (mg P_{ols} kg⁻¹ dry soil) based on a P-target of 10 mg P_{ols} kg⁻¹. In 4a-b coloration becomes lighter as iron concentration (mg Fe_{tot} kg⁻¹ dry soil) decreases. Data points with a P_{ox} -concentration over 1.5g P_{ox} kg⁻¹ dry soil or a Fe_{tot} -concentration over 50g Fe_{tot} kg⁻¹ dry soil were removed to make coloration distinguishable. In 4c-d coloration becomes more yellow as the P_{ols}/P_{ox} -ratio increases. Data points with a P_{ols}/P_{ox} -ratio over 1 were removed to make coloration distinguishable. The line at 25 mg P_{ols} kg⁻¹ in 4b and d indicates the transition from P-mining (at higher P_{ols} -concentrations) to mowing (at lower P_{ols} -concentrations).