P-removal for restoration of *Nardus* grasslands on former agricultural land: cutting traditions

Running head (3-6 words):

Restoring P-levels of post-agricultural grasslands

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Abstract

Past intensive land use complicates the successful restoration of oligotrophic species-rich grassland types. One of the major bottlenecks are the elevated nutrient levels due to fertilization, especially residual phosphorus (P). Aiming to deplete nutrients, managers often reintroduce traditional haymaking management, sometimes combined with grazing. Here, we evaluate whether this technique restores the abiotic and biotic boundary conditions for restoration of *Nardus* grassland. Seven grasslands were selected in Flanders, Belgium, which had elevated nutrient levels after the cessation of intensive agriculture 16 to 24 years ago, and which have been mown and grazed since. We compared soil and vegetation data of these post-fertilization grasslands with 34 well-developed oligotrophic *Nardus* grasslands. Mowing and grazing did not cause community composition to resemble that of *Nardus* grassland. Furthermore, bioavailable P-concentrations were significantly higher in the post-fertilization grasslands and P-limitation was not obtained. Restoring P-poor soil conditions through continued mowing and grazing management would take at least decades. Phosphorus-mining can shorten the restoration time by increased P-removal. Given our results, we propose a decision framework to aid planners and managers in their choice of interventions. Cost-effective efforts for restoration should be well-prepared including measurements of important initial soil characteristics. This allows for an evaluation of “distance to target” and the selection of an effective restoration technique. These techniques may involve cutting with mowing tradition and utilizing P-mining or topsoil removal.

Keywords

Abiotic ecological restoration, bioavailable phosphorus, mowing and grazing, P-mining, semi-natural grassland, topsoil removal

Implications for Practice

- Efforts to restore species-rich grasslands on former fertilized agricultural land with mowing and hay removal are not always successful, both in an abiotic and a biotic perspective.
- Phosphorus-removal with P-mining is faster than with mowing, but biotic restoration is postponed for a quite long timespan.
- We advise practitioners in ecological restoration to focus their efforts on realistic targets and select the appropriate restoration fields and techniques. It might be most efficient to invest some money in the abiotic screening of parcels before purchasing them.

Introduction

Globally, ecosystem restoration has become an important tool to stop biodiversity loss (Aronson & Alexander 2013). Within the European Union, the habitats directive appointed several semi-natural species-rich grassland habitat types with a priority for ecological restoration (Habitats Directive 92/43/EEC). Effective grassland restoration management requires correct identification of threatening processes, understanding of the underlying ecological mechanisms that can influence successful restoration, and recognition of appropriate interventions for a given context (Perring et al. 2015). In the case of semi-natural grasslands, the main threats are abandonment of traditional management, i.e. haymaking once or twice a year and/or extensive grazing, and land use intensification. After abandonment, forests gradually establish and lower the grassland species diversity (Hansson & Fogelfors 2000). Here, reinstating traditional management with or without species reintroductions could be a sufficient restoration strategy (e.g. Winsa et al. 2015). But, land use intensification is a more severe threat (Hooftman & Bullock 2012; Middleton 2013), especially if the land has been fertilized excessively (Gough & Marrs 1990; Walker et al. 2004). Fertilization increases grassland productivity and changes the competitive interactions between species, which can cause shifts in plant community composition and lead to biodiversity loss (Harpole & Tilman 2007; Hautier et al. 2009).

It has been debated whether nitrogen (N) or phosphorus (P) fertilization was the most important driver for this loss of plant biodiversity in grasslands. Ceulemans et al. (2013) concluded that P-fertilization presents a greater threat to biodiversity. This is confirmed in other studies (Janssens et al. 1998; Olde Venterink et al. 2003) and, also both P-limited and NP-co-limited grasslands appear to contain more endangered rare plant species than N-limited grasslands (Wassen et al. 2005). This evidence suggests that grassland restoration on former fertilized land should aim at reducing the nutrient content, especially soil-P.

However, lowering the soil-P content is not straightforward because P is one of the least mobile mineral nutrients (Stevenson & Cole 1999) and P-fertilization legacies can last from decades to millennia (Dupouey et al. 2002; McLauchlan 2006). Soil-P can be conceptualized as occurring in three pools differing in bioavailability (De Schrijver et al. 2012): (i) bioavailable P, which is available for plant-uptake within one growing season; (ii) slowly cycling P, which can become available for plant-uptake in the long-term as it can replenish the bioavailable P-pool; and, (iii) occluded P, which is assumed to be unavailable for plant-uptake. Phosphorus-uptake by plants and, hence P-limitation, are closely linked with the bioavailable P-pool (Gilbert et al. 2009). But to decrease this bioavailable P-pool, also slowly cycling P needs to be considered (van Rotterdam et al. 2012).

The soil-P content can be decreased by removing P taken up by plants. The P-removal rate depends upon the amount of harvested biomass and upon its P-concentration. In case of grassland restoration on fertilized land, the practice of mowing with hay removal after the cessation of fertilization is often used, sometimes combined with grazing. Mowing removes N effectively because nitrate is mobile and highly susceptible for leaching or plant-uptake (Storkey et al. 2015). However, mowing does not sufficiently decrease the soil-P-content on heavily fertilized agricultural land (Smits et al. 2008). The reason for low annual P-removal with mowing is the declining biomass production due to limitation of other nutrients than P, namely N (Van Der Woude et al. 1994; Smits et al. 2008) and/or potassium (K) (Oelmann et al. 2009). Restoring P-poor soil conditions through mowing may consequently take a long time and mowing can, therefore, fail –as a single measure– to restore biodiversity on heavily fertilized land (Smits et al. 2008).

Another technique for decreasing soil-P content, suggested by Marrs (1993) and Crawley et al. (2005), is P-mining. Herewith, P-removal is maximized by cultivating crops or grass whereby biomass production is optimized by fertilization with growth-limiting nutrients, other than P. Mainly addition of N and K is needed to keep biomass production high. In the first phase of P-mining, the bioavailable P-pool is constantly and sufficiently replenished from the slowly cycling P-pool (Vanden Nest et al. 2015). Later in the P-mining process, P usually becomes depleted in the rhizosphere, limiting plant-growth and, consequently, P-removal (Koopmans et al. 2004). This complicates estimations of P-removal along the restoration process.

Here, we study the restoration success of seven post-fertilization grasslands that have been mown followed by grazing for more than 15 years in order to restore *Nardus* grasslands. To get insight in the 'distance to target', of the post-fertilization grasslands, we compare their vegetation composition and soil-P concentrations with well-developed *Nardus* grasslands. We measured how much P has been removed by the practice of mowing and calculated the P that would have been removed by a management of P-mining. The effectiveness of both mowing and P-mining techniques was assessed by calculating the time needed for restoration. Finally, we created a decision model that helps managers to select the most time- and cost-efficient restoration technique.

Methods

Field measurements in Nardus grasslands and post-fertilization grasslands

We focus on restoration of acidophilous lowland *Nardus* grasslands in the Atlantic zone (European Priority Habitat Type 6230). These dry or mesophile perennial grasslands are semi-natural, and need extensive management, i.e. mowing or grazing management to halt succession towards forest (Galvánek & Janák 2008). Further, *Nardus* grasslands are oligotrophic (De Graaf et al. 2009) and typical species include *Nardus stricta* L.*, Danthonia decumbens* (L.) DC.*, Veronica officinalis* L. and *Potentilla erecta* (L.) Räuschel (Appendix S1).

To assess vegetation composition and soil data of well-developed *Nardus* grasslands, we used a database containing data of 34 parcels spread over 11 locations in Flanders, Belgium (INBO 2015). The plant species cover was measured in a 9 m^2 quadrat per parcel in July - September 2012 – 2014. In each *Nardus* grassland, one representative quadrat was selected without using the presence or absence of target plant species as a selection criterion. Subsequently to these vegetation surveys, nine soil cores (0-10 cm) were collected in each quadrat and combined into one sample (0.5 L). These samples were dried (40°C for 48 h), sieved (2 mm sieve size) and chemically analysed (see further chemical analyses).

We selected seven post-fertilization grasslands, all with comparable hydrology and soil texture as the Nardus grasslands. These grasslands were located on relatively dry, sandy soils (Podzol in WRB classification) in two neighbouring nature reserves in northern Belgium: Turnhouts Vennengebied and Landschap de Liereman (Appendix S2). The seven grasslands were in intensive agricultural use, and hence, fertilized until 16 – 24 years ago. Since then, although still exposed to atmospheric N-deposition (31 kg N

ha^{-1} y^{-1}; Cools et al. 2015), active fertilization has ceased. The management consisted of mowing with hay removal once a year in July and grazing in late summer with ponies or cows.

Vegetation measurements were performed in July 2014 in 4m² plots per parcel, the number of plots per parcel (two to six) depended on the size and the heterogeneity of the grassland. Four soil cores (0-10 cm) were collected from each quadrat and combined into one sample (0.3 L). The samples were dried, sieved and analysed for bioavailable and slowly cycling P (see further chemical analyses).

In the post-fertilization grasslands, P-removal by the current mowing practice was assessed by measuring biomass production and biomass P-concentration (P_{DM}) in one 0.25 m^2 subplot within each of the quadrats. The sward was cut two cm above the soil level on the same day as the vegetation survey. Vegetation samples were dried (70°C for 48h), weighed to obtain the total dry biomass (DM) and ground before chemical analysis (see further).

Chemical analyses

As a measure for the bioavailable P-pool (Gilbert et al. 2009), soils were extracted in $NaHCO_3$ (P_{Olsen}) following ISO 11263:1994(E). In order to get insight in the slowly cycling P pool, we extracted soils in ammonium-oxalate-oxalic acid ($P_{Oxalate}$ according to NEN 5776:2006; van Rotterdam et al. 2012). Extracted P was measured colorimetrically according to the malachite green procedure (Lajtha et al. 1999). $P_{Oxalate}$ concentrations were not available in the database of *Nardus* grasslands and were therefore calculated based on the P_{Olsen} concentrations. We assessed the relation between P_{Olsen} and $P_{Oxalate}$ in 120 soil samples taken in close vicinity to the post-fertilization grasslands. Linear regression analyses revealed a strong relation between P_{Olsen} and $P_{Oxalate}$: $P_{Oxalate} = 0.67 + 3.03 * P_{Olsen}$; $R^2 = 0.93$ and $p<0.001$ (*lm* function in the R package *stats*; Fig. 1). Additionally, soil-pH_{H2O} in the post-fertilization grasslands is presented in Appendix S2.

Plant biomass was analysed for total P concentration (P_{DM}) by digesting 100 mg of sample with 0.4 mL $HClO_4$ (65%) and 2 mL HNO_3 (70%) in Teflon bombs for 4 h at 140°C. P was measured colorimetrically according to the malachite green procedure (Lajtha et al. 1999), and total K concentration (K_{DM}) by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS). Total nitrogen concentration (N_{DM}) was measured by high-temperature combustion at 1,150°C using an elemental analyzer (Vario MACRO cube CNS, Elementar, Hanau, Germany).

Calculations and statistical analyses

We combined two datasets of vegetation surveys differing in plot size (Nardus grasslands of 9 m^2 and post-fertilization grasslands of 4 m^2 size). To be able to compare plant species richness and vegetation composition in *Nardus* and post-fertilization grasslands, we used rarefaction curves to convert the 4 m^2 quadrats in post-fertilization grasslands into one 9 m^2 quadrat per grassland, a procedure made possible by the multiple quadrats per post-fertilization grassland (Appendix S3, and see Gotelli & Colwell 2001).

We tested for differences in the number of plant species and typical species (Appendix S1) between *Nardus* grasslands (n=34) and post-fertilization grasslands (n=7) with *t.test* in the R-package *stats* without equal variances (p<0.05; R Core Team 2015). To explore potential differences in plant community composition, we performed a non-metric multi-dimensional scaling analysis (NMDS) with *metaMDS* from R-package *vegan*. We used the Lennon dissimilarity index to quantify between-plot compositional differences with the original quadrat data. Since this index is derived from species turnover only, it excludes nestedness patterns derived from richness differences (Baselga 2010). Convex hulls were added with *ordihull* from R-package *vegan* (Fig. 2; Oksanen et al. 2016). We performed a permutational analysis of variance on the same dissimilarity matrix, with grassland type as predictor and a significance based on 999 permutations (Anderson 2001). Plant species significantly indicative for either *Nardus* or post-fertilization grasslands from the quadrats recorded here were obtained by indicator value analysis with *multipatt* in the R-package *indicspecies* (De Cáceres et al. 2010).

We tested for differences in soil P_{Olsen} and $P_{Oxalate}$ concentrations between *Nardus* grasslands (n=34) and post-fertilization grasslands (n=7) by using *t.test* in the R-package stats without equal variances. To get insight in the abiotic 'distance to target' of the post-fertilization grasslands, we used a threshold value for bioavailable P of 12 µg P_{Olsen}/g soil, which was calculated as the 95 percentile of the dataset gathered in Raman et al. (Instituut voor Natuur- en Bosonderzoek, Brussel, unpublished data). This value corresponds with a slowly cycling P-pool of 37 µg $P_{Oxalate}$/g when converted with the linear regression as discussed previously.

P-oxalate stocks in the 0-10 cm soil layer were calculated by assuming soil bulk density of 1.4 g/cm^3 for sandy soils. For Nardus grasslands, a threshold value of 51.8 kg $P_{Oxalate}$/ha was herewith calculated. Subtracting this threshold value from the P-oxalate stocks of each post-fertilization grassland gave insight in the 'distance to target', i.e. the excess of slowly cycling P ($P_{Excessive}$).

The annual P-removal by mowing the post-fertilization quadrats was calculated by multiplying DM with P_{DM}. For each post-fertilization grassland, we estimated how many years it would take to reach the threshold of *Nardus* grassland by dividing $P_{Excessive}$ with its current annual P-removal.

How long it takes to reach the threshold value depends both on the P-concentrations in the soil and the soil depth in which concentrations are elevated. When parcels are regularly ploughed, it is likely that P-concentrations are elevated in the complete furrow, or even deeper when soils were P-saturated and P-leaching occurred. If no regular ploughing has occurred, sometimes only the topsoil has elevated P-concentrations. We illustrated this issue by performing our calculations for two cases: i) case in which only the 0-10 cm topsoil and ii) case in which the furrow (0-30 cm soil) contains elevated P-concentrations. The P-concentration for the 0-30 cm soil was assumed to be the same as in the 0-10 cm soil. We here want to stress that these calculations are only estimations, as we here assume P-removal by mowing to stay constant in time. It might, however, be possible that annual P-removal would further decrease as a consequence of P-depletion in the soil (Schelfhout et al. 2015).

To verify whether the target of P-limitation or NP-co-limitation was obtained in the post-fertilization grasslands (see Introduction), we compared P_{DM} and N_{DM} to the ecological critical thresholds of P- and N-limitation in grasslands (P_{DM}<0.7 mg P/g and N_{DM}<14 mg N/g according to Wassen et al. 1995; Güsewell 2004). To verify which nutrient(s) limited biomass production for P-mining purposes, we compared P-, N- and K-concentrations in plant biomass to agricultural standards (P_{DM}<2.6 mg P/g, N_{DM}<20 mg N/g, K_{DM}<20 mg K/g; Bailey et al. 1997).

For each post-fertilization grassland, we calculated potential P-extraction by P-mining. P-mining results in lifting N- and K-limitation through fertilization, and we assume biomass production will increase compared to the mowing management. Due to NK-fertilization, P-removal will increase with biomass production, while P_{DM} will probably not change, as it is mainly influenced by bioavailable soil-P-concentrations (Gilbert et al. 2009).

We furthermore modelled how much time it will take to deplete $P_{Excessive}$ by P-mining. Also here we want to stress that our calculations are only rough estimations because little experimental knowledge is available on how effective P-mining is in the long-term (MacDonald et al. 2012). During P-mining management, annual P-removal declines over time with decreasing soil P-bioavailability (Schelfhout et al. 2015). Therefore, we assume that initially, in a soil with high bioavailable P-concentration (>65 µg P_{Olsen}/g), annual P-removal is high (i.e. 45 kg P/ha according to unpublished results on P-mining fields in close vicinity to the post-fertilization grasslands). Further, we assumed P-mining to slow down until 20 µg P_{Olsen}/g is reached in steps according to Schelfhout et al. (2015): 65-55 µg P_{Olsen}/g, 33.5 kg P/ha; 55-36 µg P_{Olsen}/g, 22 kg P/ha; 36-25 µg P_{Olsen}/g, 14 kg P/ha; 25-20 µg P_{Olsen}/g, 10 kg P/ha. When bioavailable P-pools are depleted any further, P-removal by P-mining will likely approach P-removal by mowing. Therefore, we suggest changing the management from P-mining to mowing without NK-fertilization when a bioavailable P-concentration of 20 µg P_{Olsen}/g is achieved. In this last step, we use the measured P-removal by mowing from each post-fertilization quadrat until the target of 12 µg P_{Olsen}/g is reached. Also these calculations were performed for two soil depths, 0-10 cm and 0-30 cm.

Results

The post-fertilization grasslands were species-poorer than the observed *Nardus* grasslands: we found on average only eight vascular plant species per 9 m^2 in post-fertilization grasslands in contrast to on average 22 vascular plant species per 9 m^2 (Table 1) in *Nardus* grasslands. In the post-fertilization grasslands, no typical *Nardus* grassland species were found, whilst the *Nardus* grasslands had on average four typical species. Furthermore, we found that plant communities of post-fertilization and reference grasslands did not resemble each other (Fig. 2).

The bioavailable and slowly cycling P-concentrations were significantly higher in the post-fertilization grasslands (Fig. 3; Table 1) compared to the *Nardus* grasslands. While the *Nardus* grasslands had very low bioavailable P-concentrations (1.5 – 14.1 µg P_{Olsen}/g), concentrations in our post-fertilization grasslands ranged between 25 and 114 µg P_{Olsen}/g; i.e. 1.8 to 13 times higher concentrations than the calculated threshold for *Nardus* grasslands (12 µg P_{Olsen}/g, Raman et al., Instituut voor Natuur- en Bosonderzoek, Brussel, unpublished data). We estimated that on average 170 to 511 kg $P_{Oxalate}$/ha, dependent on the soil depth with elevated P-concentrations, should be removed from the post-fertilization grasslands to reach the bioavailable P-threshold of 12 µg P_{Olsen}/g (Table 2).

We compared N, K and P concentrations in the plant biomass of the post-fertilization grasslands to agricultural standard values (growth limitation when N_{DM}<20 mg N/g, K_{DM}<20 mg K/g, P_{DM}<2.6 mg P/g, Bailey et al. 1997). In five of the seven post-fertilization grasslands, we found a limitation of N and P, while K was limiting in all seven (see Fig. 4). A review on nutrient limitation by Güsewell (2004) revealed that an N:P ratio of less than 10 indicates that biomass production will be stimulated by N-fertilization. We found that the N:P ratio was less than 10 in all post-fertilization grasslands, and in none of them the ecological P-limitation was reached (P_{DM}<0.7 mg P/g, Wassen et al. 1995).

The current mowing management in the post-fertilization grasslands annually removes on average 1.9 ton DM/ha and 5.3 kg P/ha. We estimated that it would take about 40 to 118 years to reach the soil P threshold with this P-removal rate, the range dependent of the soil depth with elevated P-concentrations (Table 2). Our calculations show that with P-mining, the time needed to reach the restoration target is less than half of the time needed with mowing management (on average 14 to 46 years, depending on the depth of soil with elevated P-concentrations, see Table 2).

Discussion

Mowing and grazing of post-fertilization grasslands for more than 15 years did not lead to plant communities that resembled the *Nardus* grasslands. Species-richness was significantly lower in the studied post-fertilization grasslands and typical *Nardus* species were absent. Further, P-limitation in plants, according to ecological thresholds, was not obtained, while it is an objective for restoring species-rich grasslands (Ceulemans et al. 2013). Also, compared to the threshold of 12 µg P_{Olsen}/g for *Nardus* grasslands (Raman et al., Instituut voor Natuur- en Bosonderzoek, Brussel, unpublished data), the bioavailable soil P concentration in the post-fertilization grasslands was typically more than five times higher. Restoration efforts to a target of species-rich grasslands may thus be compromised by high residual soil fertility. Our small case study illustrates a gap between theory and practice: i.e. practitioners reinstating traditional grassland management on fields with an agricultural legacy without considering the abiotic and the biotic bottlenecks.

By the technique of mowing and hay removal, it would take many decades to reach the bioavailable P-threshold. The current P-removal rate was found to be much lower in our post-fertilization grasslands than in intensively-fertilized grasslands (on average 5.3 kg P ha^{-1} y^{-1} compared to more than 30 kg P ha^{-1} y^{-1} in optimal growing conditions (Liebisch et al. 2013)). This low P-removal was due to the observed low biomass production. Intensively-fertilized grasslands (for example annually fertilized with 210 kg N/ha and 116 kg P/ha) can annually produce up to 15 t DM/ha (Liebisch et al. 2013), in contrast to the post-fertilization grasslands where less than 3 t DM/ha was removed by mowing once per year (at least in all quadrats but one, where 5 t DM/ha was removed). This finding was similar to observations by Bakker et al. (2002) in grasslands mown for 25 years without fertilization. While we can assume that our post-fertilization grasslands were previously intensively-fertilized and were, hence, very productive, the low biomass production can be explained by nutrient limitation of N- and/or K, in some cases also of P, at least according to agricultural standard values. This limitation was, however, not enough for decreasing biomass production to the level necessary for restoration of *Nardus* grasslands (0.1–0.33 ton DM/ha according to Bakker et al. 2002 and Bedia & Busqué 2013).

P-mining aims at maximizing P-removal by NK-fertilization, and our calculations showed that this technique might halve the time needed to restore the necessary P-concentrations. Although some studies are investigating P-mining in the field (Dodd et al. 2012; Postma et al. 2015), more long-term field experiments are needed to get better insight in which crops are most optimal to reach restoration targets. Managers can choose to mine as quick as possible by means of an intensive agricultural practice, using crops that are not interesting for biodiversity (e.g. corn) and crop protection products, or undertake a more extensive way of mining, with crops being more interesting for biodiversity (e.g. grass-clover) and no crop protection (Carvell et al. 2006; Goulson et al. 2011).

Little field data is available on the costs/gains of P-mining. To make the technique of P-mining economically feasible for farmers, it is important that the crop or hay quality is guaranteed. In fields with low soil P-concentrations in an agricultural context, P in forage will also likely be suboptimal and this can be the cause of a lower nutritional value. For forage to serve as the only feed component of the diet of high yielding dairy cows, it should not contain less than 3 mg P/g of dry matter (Valk et al. 1999). Possibly other less common used crops will be more optimal in the later stages of P-mining (e.g. buckwheat (Simpson et al. 2011)).

As an alternative for mowing and P-mining, one method not examined in our study that might be useful is removing the nutrient-enriched mineral soil layer immediately creates nutrient poor soil conditions (Frouz et al. 2009). It is, however, important to beforehand accurately determine the depth of the soil layer that needs to be removed (Hölzel & Otte 2003). Soil removal is an effective technique with a high one-time cost (Klimkowska et al. 2010), though probably cheaper than the cumulative cost for long-term mowing (Smolders et al. 2008). This one-time cost could be further reduced if the topsoil can be re-used, for construction of dikes, or to introduce topographic and plant compositional heterogeneity on site, as being demonstrated in Australian grasslands (Gibson-Roy & McDonald 2014).

Overcoming abiotic bottlenecks and reducing the P concentrations alone have rarely led to the targeted habitat recovery (Bischoff 2002; Poschlod & Biewer 2005). As such, biotic issues must be tackled, such as the potential unavailability of seeds of target plant species (Ozinga et al. 2009) and the general absence of a typical community of soil organisms (van der Heijden et al. 2008).

Decision model for selecting a restoration technique

We propose a decision model to aid practitioners in selecting the appropriate restoration technique given the above-mentioned bottlenecks (Fig. 5).

To obtain effective restoration management, we propose to focus on fields where targets can be reached within a reasonable timespan and cost, which can of course both highly vary. The first step is to measure relevant abiotic parameters and to compare them with the thresholds of the targeted habitat type (calculation of the 'abiotic distance to target').

If abiotic parameters do not meet the threshold values, abiotic restoration should precede biotic restoration. As such, the selection of the appropriate abiotic restoration technique depends on the distance to the target and on the context of the restoration project, among other things the project budget and timing. The amount of P to be removed will determine time needed for P-mining, the depth of the P-elevated soil layer will determine the cost of topsoil removal. The cost and time of these restoration techniques may be weighed against the importance of the site within the landscape being restored.

If no substantial amount of P needs to be removed, or if abiotic parameters have been restored, we suggest biotic restoration to start (step 2) and the appropriate grassland management (mowing and/or grazing) to be introduced (step 3). Natural colonization with target plant species can only happen if source populations of the target species occur adjacent to the restoration sites (Winsa et al. 2015). If landscape fragmentation hinders colonization, as was the case in our post-fertilization grasslands, the dispersal of species needs an active approach (Piessens et al. 2005; Helsen et al. 2013). Plant species with low dispersal capability can be introduced actively via transfer of seed or hay (Edwards et al. 2007; Hedberg & Kotowski 2010). Also, the active introduction of key species, for example the hemiparasitic genus *Rhinanthus* spp. or the hemiparasite *Pedicularis sylvatica*, can affect plant species richness in grasslands by changing the community structure and reducing the biomass of competitive graminoids (Pywell et al. 2004; Demey et al. 2015). Examples from practice show successful restoration of species-rich grasslands where the reintroduction of target species followed topsoil removal (Gibson-Roy et al. 2010; Berendse et al. 1992; Tallowin & Smith 2001; Hölzel & Otte 2003; Allison & Ausden 2004).

The post-fertilization grasslands under study clearly show that the target of species-rich *Nardus* grasslands is far from reached after at least 15 years of traditional grassland management (mowing with hay removal and grazing). We estimated that restoring P-poor soil conditions through continued mowing and grazing management may take many decades. We calculated that P-mining can significantly reduce this time period and briefly discussed the possibilities of topsoil removal.

Our results question the feasibility of striving for oligotrophic, species-rich grassland types on any given intensively-managed and fertilized agricultural parcel by using traditional mowing. Ecological restoration of semi-natural grasslands on former agricultural land might involve a large investment of time and/or money. Therefore, it is necessary for practitioners in ecological restoration to focus their efforts and select the appropriate restoration fields and the appropriate techniques. Because of the high cost, topsoil removal is more likely to be used in large restoration projects, funded by e.g. European Life+ projects. Restoration in projects with less budget, can probably better focus on fields with a history of less intensive fertilization. It might be most efficient to invest some money in the abiotic screening of parcels before purchasing them. Where restoration of oligotrophic *Nardus* grasslands is unrealistic, because of time or money issues, one could choose to develop other vegetation types nearby or adjacent to well-developed Nardus grasslands. These non-fertilized plant communities will have a composition that might not be what it should be in the short-term, but its functioning might have already been significantly improved relative to agricultural fields or grasslands, e.g. in terms of delivering food and nest sources to pollinators and other insects (Woodcock et al. 2014). Moreover, these grasslands can act as buffer zones for preventing inflow of fertilization of nearby agricultural fields.

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Tables

Table 1 Vegetation and soil properties of the post-fertilization grasslands and reference *Nardus* grasslands (mean ± SE). The number of vascular plant species in post-fertilization grasslands was estimated from rarefaction curves (Appendix S3). Slowly cycling P-concentrations in reference *Nardus* grasslands were calculated from bioavailable P-concentrations by linear regression (see Methods). Results of t-tests are indicated by the *p*-value. The list of typical *Nardus* grassland species is shown in Appendix S1

	Post-fertilization grasslands with mowing and grazing for 15 – 24 years	**Reference *Nardus* grasslands never fertilized**	***p*-value**
Number of grasslands	7	34	
Vegetation properties			
Number of vascular plant species per 9 m^2	8 ± 0.6	22 ± 1.6	<0.001
Number of typical *Nardus* grassland species per 9 m^2	0 ± 0	4 ± 0.3	<0.001
Soil properties			
Bioavailable P (µg P_{Olsen}/g)	53 ± 11	3.9 ± 0.5	0.004
Slowly cycling P (µg $P_{Oxalate}$/g)	159 ± 32	13 ± 1.4	0.004

Table 2 Biomass production and nutrient concentrations in the first and only cut in post-fertilization grasslands (n=7; mean ± SE). $P_{Excessive}$, P-removal, P-removal time to reach the maximal threshold of 12 µg P_{Olsen}/g in 0-10 cm and 0-30 cm were estimated for mowing and P-mining in the post-fertilization grasslands (n=7; mean ± SE)

	Post-fertilization grasslands with mowing and grazing for 15–24 years
Biomass production - first cut (ton DM/ha)	1.9 ± 0.4
Nutrient concentrations in biomass	
P_{DM} (mg P/g)	3.0 ± 0.2
N_{DM} (mg N/g)	19 ± 1.3
K_{DM} (mg K/g)	9.2 ± 1.1
Annual P-removal with biomass (kg P/ha)	5.3 ± 1.0
Excessive soil-P-amount to remove	
$P_{Excessive}$ in 0 – 10 cm soil layer (kg P/ha)	170 ± 45
$P_{Excessive}$ in 0 – 30 cm soil layer (kg P/ha)	511 ± 135
Estimation of time to reach threshold	
0 – 10 cm mowing (years)	40 ± 11
0 – 10 cm P-mining (years)	14 ± 2
0 – 30 cm mowing (years)	118 ± 34
0 – 30 cm P-mining (years)	46 ± 5

Figures

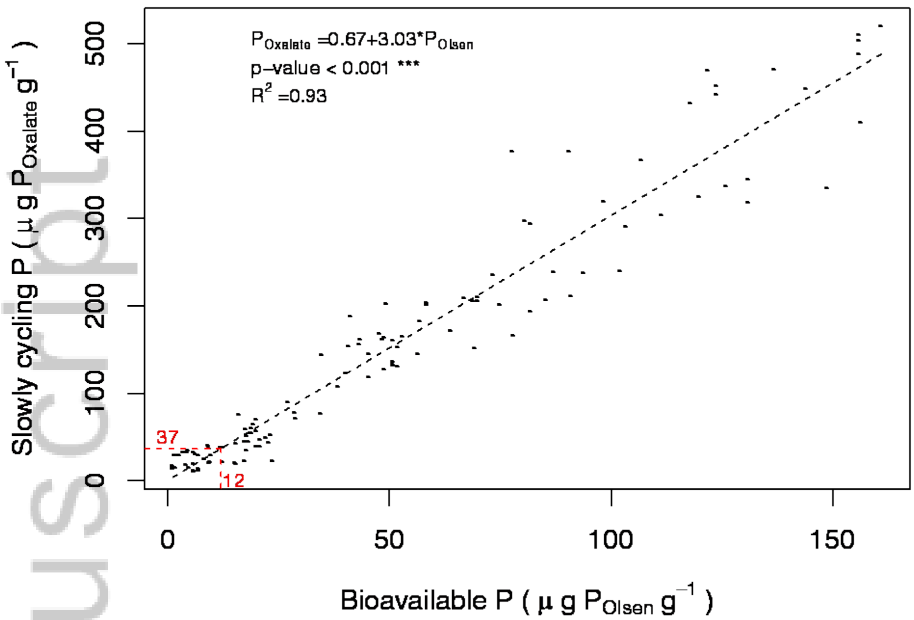

Figure 1 Linear regression between concentrations of bioavailable P (P_{Olsen}) and slowly cycling P ($P_{Oxalate}$) from 120 soil samples. The bioavailable P-threshold for *Nardus* grasslands, namely, 12 µg P_{Olsen}/g (see Methods section), corresponds to a slowly cycling P-concentration of 37 µg $P_{Oxalate}$/g and is indicated with red dashed lines

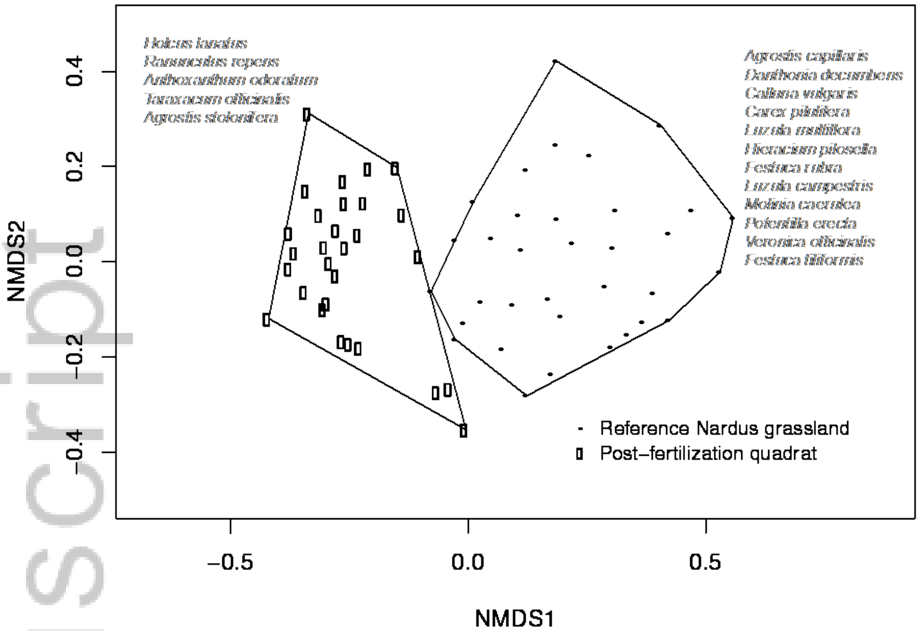

Figure 2 Results of a non-metric multi-dimentional scaling (NMDS) ordination of the plant communities in the 34 reference *Nardus* grasslands and the seven post-fertilization grasslands (29 quadrats are shown). Distances between points represent differences in the composition of vegetation plots that are derived from turnover (Lennon dissimilarity; *f*=44.2; *p*<0.001) and are independent from variation in species richness. Indicative species significantly associated with our reference *Nardus* grasslands and post-fertilization grasslands respectively, were shown respectively in the right and left corners in gray (*p*=0.001)

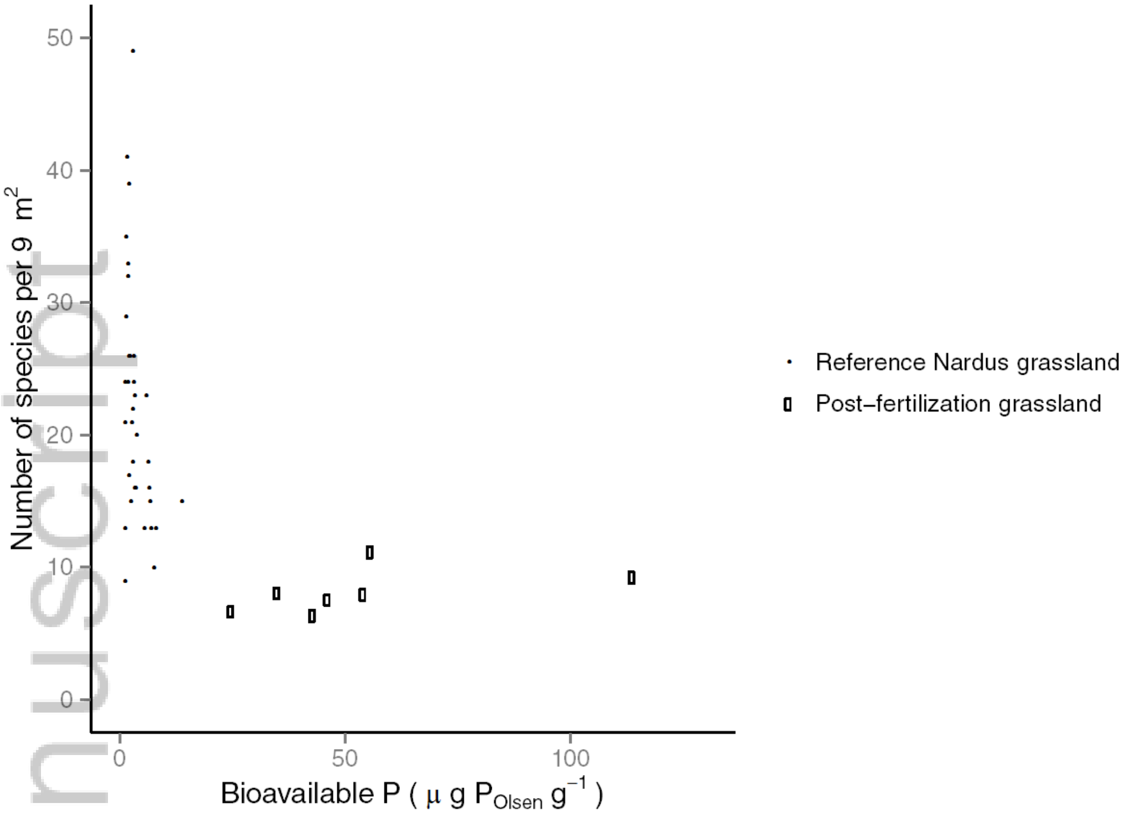

Figure 3 Number of vascular plant species found in 9 m^2 quadrats for reference *Nardus* grasslands (n=34) and post-fertilization grasslands (n=7) versus bioavailable P-concentration. Number of plant species in post-fertilization grasslands was estimated from rarefaction curves (Appendix S3)

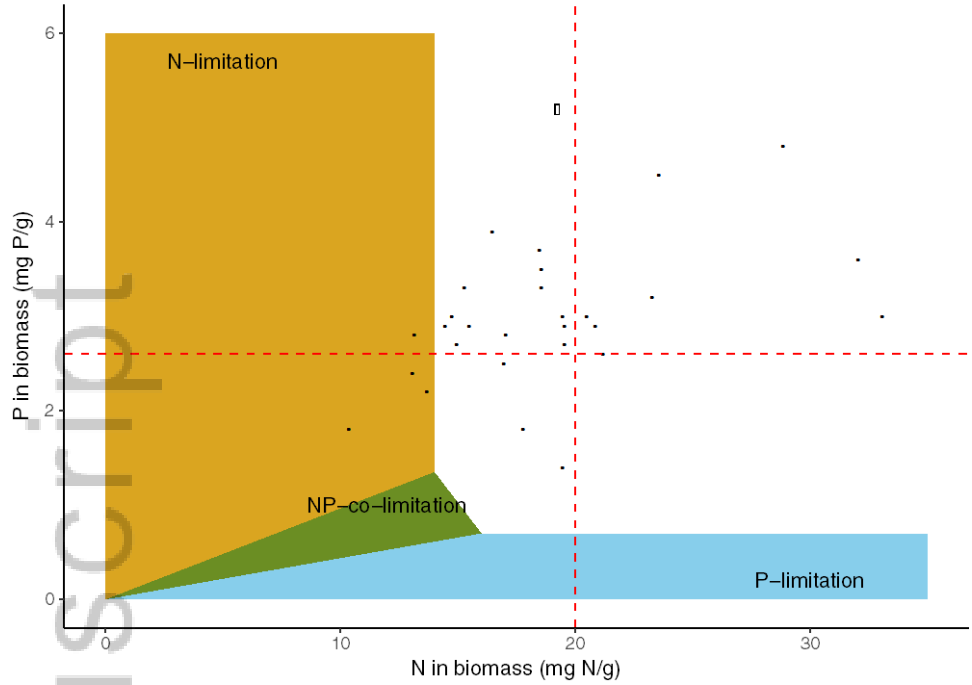

Figure 4 N_{DM}- and P_{DM}-concentration in the vegetation of post-fertilization quadrats (n=29) are shown as black dots. The coloured polygons indicate N-, P- or NP-co-limitation in an ecological context (Wassen et al. 1995). The red dashed lines indicate N- and P-limitation in an agricultural context (Bailey et al. 1997). The open circle shows the one quadrat where K was not limitating according to the agricultural standard of Bailey et al. (1997)

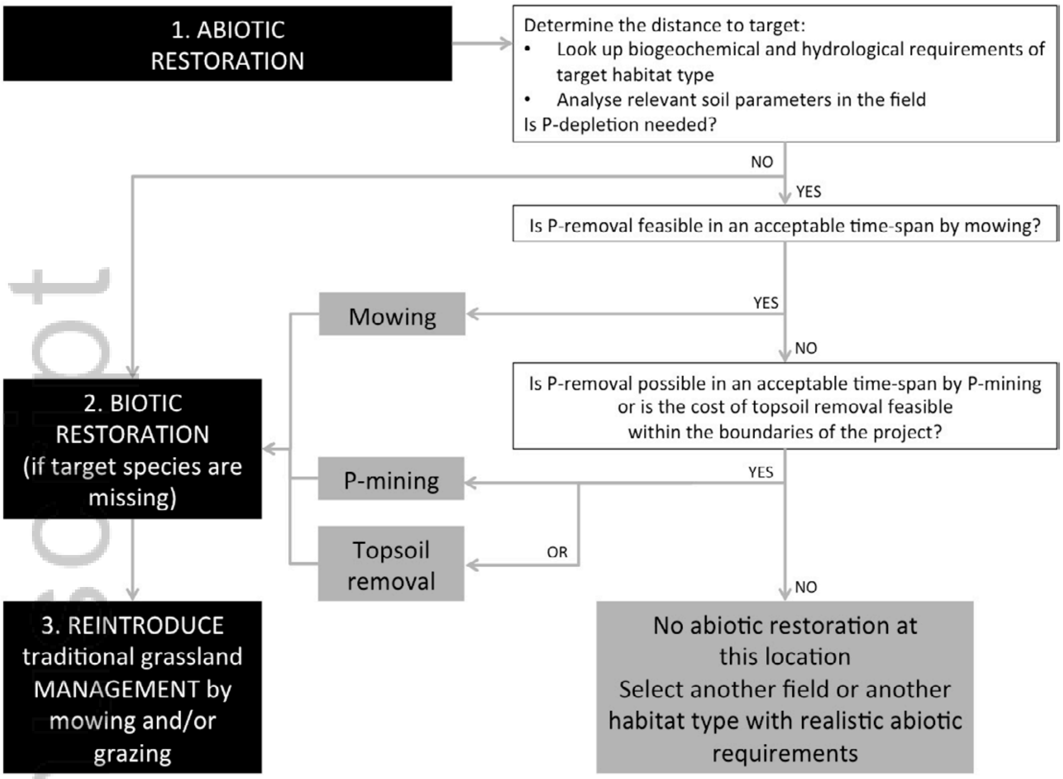

Figure 5 Decision model for practitioners restoring species-rich semi-natural grasslands on fertilized land. This decision model was inspired by Kemmers and van Delft (2010) but with a particular focus on abiotic and biotic restoration. In cases where P-depletion is needed, the choice for an appropriate restoration technique is based upon the time and cost needed for restoration. The time needed with mowing or P-mining depends upon $P_{Excessive}$ while the cost for topsoil removal is dependent on the soil depth with elevated P-concentrations