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Facilitating a regime shift from agricultural grasslands to species-rich hay meadows

A case study in the Dutch peat district

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Abstract

Intensive dairy farming in peatlands has caused a wide range of environmental problems, such as soil subsidence, biodiversity loss and eutrophication. It is desirable to facilitate a regime shift in the agroecosystem to reduce these problems. A case study was set up to determine the strategies required to facilitate a regime shift toward species-rich hay meadows in the Dutch peatland area. The initial state of typical species-poor meadows and a more species-rich meadow were compared, based on their botanical diversity and soil properties. An experiment, consisting of field treatments (mowing and tillage) and the sowing of hay meadow species, was set up to assess the effectiveness of these treatments for the introduction of the desired species.

The results of this study show that the prevailing environmental conditions are suboptimal to initiate the regime shift. Especially, the high density of *Lolium perenne*, the high soil nutrient concentrations and the low water levels are unsuitable for the desired hay meadow species. The results of the field experiment demonstrate that drastic measures need to be taken in order to successfully introduce the desired species.

To facilitate the regime shift, either a long-term strict mowing regime or topsoil removal will be required. Once soil nutrient concentrations are sufficiently low, water levels should be elevated, and species should be introduced through seed mixtures from local origin.

The findings of this study can be used in similar agroecosystem restoration projects that aim to reduce the environmental problems in the Dutch peatland area and other peatlands in northwest Europe.

Keywords: *Ecological restoration, Extensive agriculture, Peat soil properties, Species-rich hay meadows*

Abstract (Dutch)

Intensieve melkveehouderij in de westelijke veenweidegebieden heeft bijgedragen aan een breed scala milieuproblemen, zoals bodemdaling, biodiversiteitsverlies en stikstof- en fosfaat-gerelateerde problemen. Het is daarom wenselijk om een gecontroleerde verandering in het agrarische ecosysteem in gang te zetten die deze problemen zou kunnen verminderen. Een casestudie was opgezet om uit te vinden welke strategieën nodig zullen zijn om vochtig hooiland te creëren in het veenweidegebied. De huidige staat van karakteristieke soortenarme weilanden en een meer soortenrijk weiland werden vergeleken op basis van de botanische diversiteit en bodemeigenschappen. Een experiment, met verschillend beheer (maaien en verticuteren) en het zaaien van de gewenste hooilandsoorten, was opgezet om de effectiviteit van deze beheertypen voor de introductie van de soorten te evalueren.

De resultaten van deze studie laten zien dat het heersende milieu suboptimaal is voor het inzetten van de gewenste verandering in biodiversiteit. Met name de hoge grasdichtheid, de hoge voedselrijkdom van de bodem en de lage grondwaterstanden zijn ongeschikt voor de gewenste hooilandsoorten. De resultaten van het veldexperiment laten zien dat hevige maatregelen nodig zijn om de gewenste soorten met succes te kunnen introduceren.

De uiteindelijke systeemverandering kan alleen worden verkregen wanneer een streng en langdurig maairegime wordt opgezet of wanneer de toplaag van de bodem zal worden afgegraven. Zodra de voedselrijkdom van de bodem laag genoeg is, zal de grondwaterstand moeten worden verhoogd, en kunnen de hooilandsoorten worden geïntroduceerd met behulp van een lokaal zaadmengsel.

De bevindingen van dit onderzoek kunnen van toepassing zijn in vergelijkbare restoratieprojecten met betrekking tot het verminderen van de milieuproblemen in het agrarische ecosysteem van veenweidegebieden in Nederland en de rest van noordwest Europa.

1 Introduction

Globally, agricultural practices have contributed to environmental problems such as biodiversity loss, water pollution, soil acidification, air pollution and climate change (Leip et al., 2015; Poore & Nemecek, 2018). Particularly, animal husbandry has detrimental effects on the environment (Erisman & Slobbe, 2019; Leip et al., 2015; Van Beek et al., 2004). In peatlands across northwest Europe, intensive agriculture has caused severe environmental problems. For instance, in the Netherlands, peat soils have been drained for centuries to enhance the soil's load-bearing capacity for cattle and heavy machinery, and to provide additional nitrogen for crop production (Boxem & Leusink, 1978; Schothorst, 1977). Initially, this has been beneficial to agriculture, but eventually the peatlands remained too wet to support arable farming. Moreover, soil subsidence, the emission of carbon dioxide (CO_2) and loss of botanical richness are the negative side effects of draining these peatlands (Erkens et al., 2016; Strien & Melman, 1987).

Today, the Dutch peatland area is dominated by species-poor livestock meadows, which require continuous drainage and high input of fertilizers (Boxem & Leusink, 1978; Schippers et al., 2019). Ongoing subsidence, caused by drainage, is associated with the emission of CO_2 that contributes to climate change (Erkens et al., 2016). Fertilizer application interferes with the nitrogen and phosphorous cycles, which has both environmental and societal implications (Erisman & Slobbe, 2019; Leip et al., 2015; Van Beek et al., 2004). For instance, loss of nutrients to the water system leads to eutrophication and deterioration of drinking water, and emissions of ammonia and nitrogen oxides affect air quality and human health (Leip et al., 2015). Moreover, livestock farming is considered responsible for population decline of meadow birds, such as the black-tailed godwit (*Limosa limosa*), northern lapwing (*Vanellus vanellus*) and common redshank (*Tringa totanus*) (IUCN, 2021; Kleyheeg et al., 2020). If no drastic measures regarding land

Box 1. *Species-rich hay meadows*

Historically, hay meadows are species-rich (flowery) grasslands that would develop in fields that were only used for haymaking. In the peatland areas, these meadows were often too wet to support cattle. Because of continuous swath removal and the lack of nutrient inputs, a low-productive system would develop.

A hay meadow system can be characterized by species that are highly dependent on particular soil properties and moisture levels. The number of species can vary between 20 and 40 species per 25 m^2. In the Dutch peatland area, typical hay meadow species are *Caltha palustris*, *Rhinanthus angustifolius* and *Silene flos-cuculi*. A full list of hay meadow indicator species can be found in the Appendix A.

Species-rich hay meadows require a water level of 20-30 cm below ground level and (bi)annual mowing with swath removal. To maintain the hay meadow vegetation, the maximum nitrogen input may be 25 kg per year. Eventually, this agroecosystem generally provides a yield of 3-6 tonnes dry matter per hectare.

(BIJ12, 2017; Schippers et al., 2019)

management and organisation are taken in the near future, it is expected that meadow bird populations will continue to decline and might even go extinct locally (Melman & Sierdsema, 2017). Overall, the impacts of intensive dairy farming in the Dutch peatland area inhibit the provision of important ecosystem services, and it is evident that the prevailing agricultural system is unsustainable in this area (Erisman & Slobbe, 2019; Smit et al., 2012).

A reduction of the main environmental problems could potentially be induced by raising the water levels and reducing the use of fertilizers, which could give rise to the development of a so-called hay meadow system (Box 1). Compared to the typical agricultural grasslands, this agroecosystem, that relies on wetter and less nutrient-rich conditions, could support a higher biodiversity and provide more ecosystem services, i.e. carbon sequestration, water regulation and nutrient cycling (Ritzema et al., 2016). Moreover, the hay meadow system can still provide agricultural production, which implies that this ecosystem could potentially become a profitable system as well.

Although there have been various studies and field experiments regarding grassland restoration (Horrocks et al., 2016; Losvik & Austad, 2002; Pavlů et al., 2021; Van der Hoek, 2005), it remains unclear how to successfully create a hay meadow system in the severely degraded peat grasslands, because of the many ecosystem properties and processes involved. Besides, it is well-known that the intensively used peat soils have accumulated large amounts of nutrients, which (especially in combination with higher water levels) could lead to the development of an undesired botanical composition (Van de Riet et al., 2014). To avoid dominance of undesirable species, such as *Juncus effusus* and *Phragmites australis*, it would be wise to keep maximal control on the envisaged regime shift from the current system towards a hay meadow system. This requires insight into the initial state of the ecosystem, in terms of botanical composition and underlying soil properties, and into the effects of management on both these factors.

The aim of this research was to clarify (1) what the initial state (baseline situation) of the ecosystem is, (2) which ecosystem properties underpin the state of the current ecosystem and the desired hay meadow system, and (3) if simple management practices could be used to create the desired system. Therefore, the baseline situation of various (agro)ecosystems in the Dutch peatland area were compared to gain insight into the required alterations of ecosystem properties, and various management strategies were tested in the field. Ultimately, the results of the analyses were used to determine the most effective strategies to facilitate the regime shift from severely degraded agricultural grassland towards a species-rich hay meadow system.

2 Materials and Methods

2.1 Study area

This study focused on the agricultural peatlands near the city of Leiden (52°2' N, 4°5' E), which are fairly representative for the general peat landscape. Research was done in three polders: the Lakerpolder (LP), Boterhuispolder (BHP) and Vrouw Vennepolder (VV). In the northern part of VV lay the target area (LvO, Box 2). Some fields that were still managed by the farmer (SVV) were located in the southern part of VV. Management of the areas differed in terms of fertilization and mowing and grazing regimes.

2.1.1 Previous & current management

LP has been managed as a nature reserve (drained by mill, no fertilization, no grazing, only mowing after the meadow bird breeding season) since a few decades by the State Forestry Commission. BHP and LvO had been used for conventional farming. BHP and LvO have been under an intensive management regime with regular mowing and heavy fertilization by injection of liquid slurry. Both polders were grazed alternately by sheep and cattle. In LvO, this management regime was ceased in December 2020, after which no fertilizers were applied, and extensive grazing was applied between April and June 2021. Fields in SVV were used for organic farming and were grazed by dairy cows and sheep. Here, swath was not removed after mowing. Organic fertilizer was applied with light-weight machines.

Box 2. *Land van Ons*

The Land van Ons cooperative is a Dutch citizens' initiative that buys agricultural land for the restoration of biodiversity in the Netherlands. It has acquired agricultural land scattered throughout the Netherlands. The Vrouw Vennepolder, near the city of Leiden, is one of their recent acquisitions. In a large part of this polder (33.2 ha) change of land use is intended, to improve biodiversity and reduce subsidence- and nutrient-related problems. In collaboration with research institutes, such as Leiden University, Land van Ons aims to set up long-term research to gain insight into sustainable use of their agricultural land.

https://landvanons.nl/

2.2 Determining baseline situation

The current state of the agroecosystem was assessed based on a selection of ecosystem properties (EP), including vegetation and invertebrate diversity and various soil properties. All EP data was collected from predetermined locations from 4 fields in LP, 4 fields in BHP, 10 fields in LvO and 4 in SVV (Appendix B). Across each field, 3 points were spread diagonally, resulting in 66 locations for sampling and recording.

Both plant and invertebrate diversity properties, as well as soil carbon (C), nitrogen (N), phosphorous (P) and pH had already been recorded in LP, BHP and LvO in September 2020. Botanical diversity was based on cover percentage per species per 1 m^2 plot. Invertebrates were counted, to obtain total number of individuals per plot and alpha diversity. Chemical soil properties were obtained from the top layer only. Additional soil data was acquired by taking new soil samples from deeper layers in LP, BHP, LvO and SVV in spring 2021.

2.2.1 Grassland phases

The total number of species per plot was determined from the September 2020 data. With the use of the vegetation data, each plot was classified based on species composition (detailed methodology in Appendix C1). The classes (0-4) were based on the grassland phases as described by Schippers et al. (2019). Phase 0 would correspond to a typical high productive grassland (>10 tonnes of dry matter per hectare), with a cover of more than 50% *Lolium perenne*. Phases 1-4 indicated lower production and generally higher species diversity. A species-rich hay meadow would correspond to phase 4 in this classification system.

2.2.2 Soil sampling

With the use of an Edelman auger (Ø 9 cm), multiple samples from the same hole were taken until the samples contained some intact peat (containing visible plant material) or until the maximum depth of the auger was reached (approx. 120 cm). Samples were placed next to a ruler to measure the depth of the various soil layers (i.e., topsoil, degraded peat and intact peat). From these samples, three separate sub-samples were taken, from a depth of 10-20 cm, 35-45 cm and 60-70 cm, for further chemical analysis. From each depth three scoops were taken with the use of a 15 ml spoon and stored in airtight bags. Bags were stored in a refrigerator until the samples were dried, for 75 hours at 70 degrees Celsius, to determine soil moisture content. A loss-on-ignition analysis (LOI) was done by the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen to determine the soil organic matter (SOM) content of each layer.

Soil chemical properties were already analysed (by NIOO-KNAW) in soil samples taken from the top layer in September 2020. The chemical properties included were acidity (pH, based on H_2O extraction), the carbon-to-nitrogen ratio (C:N, based on soil C% and soil N%), and plant available phosphorus (P in mg L^{-1} and mg kg^{-1}, based on $CaCl_2$-extraction).

2.2.3 Water levels

Groundwater levels were estimated, based on the water level of the main water system, as measured by the waterboard at 17 June, 2021 (Waterboard of Rijnland, 2021). Ground level was based on the Dutch digital elevation map (AHN3). Both variables were expressed in the Normal Amsterdam Level (NAP). To estimate the depth of groundwater beneath the soil surface, the difference between the water level and ground level was calculated using the Raster Calculator in ArcGIS.

2.3 Vegetation management experiment

A field experiment was set up to test some basic management practices that could initiate the desired regime shift. The goal of this experiment was to gain insight into the combined effect of prevailing environmental factors and the management options. Three fields, with different environmental characteristics, were set up. Two fields were located in BHP, one with moderately wet soil and a species-poor plant community (BHP-1) and one with a wetter soil and species-rich plant community (BHP-2). The third field (LvO-3) was set up in the target meadow, where the soil was relatively dry, and the plant community was moderately species-rich. In all fields, 12 plots (3.5 x 3.5 m) were set up for 4 treatments with 3 replicates each (schematic overview in Appendix C2). The treatments applied were only mowing (M-), mowing and sowing (M+), mowing and tillage (MT-), and mowing, tillage and sowing (MT+). Mowing was done with a grass trimmer and swath was removed with the use of a rake. A lawn scarifier was used for superficial tillage. Sowing was done with a species-rich hay meadow seed mix (Appendix C3) that was sourced locally and directly from nature (Biodivers BV, 2016). The mixture consisted of herbaceous species, that should have been able to develop in the still relatively nutrient-rich environment. The mixture (approx. 1 g seed per m^2) was sown by hand within 24 hours after mowing at all designated plots.

Development of vegetation was assessed over time. In the middle of each plot, a 1 m^2 quadrat was used to estimate cover percentage of all plant species present. This assessment was done before mowing in May (t0), and 9 weeks after sowing in July (t2).

2.4 Data analysis

To analyse differences in invertebrate species richness and plant species numbers between the four areas, a one-way ANOVA, with TukeyHSD post-hoc test, was used. Analyses were done in R (version 4.0.5).

Plant species numbers and soil data of the baseline assessment were processed in ArcGIS (version 10.7.1), using the interpolation tool 'Kriging', to create prediction surface maps of the EP. Soil chemistry data acquired in September 2020 could only be interpolated for the LvO area, since no data was collected in SVV. Soil data of spring 2021 could be interpolated for the entire study area that was defined within VV. Each Kriging analysis was executed for each polder separately to rule out any effects of data measured in other polders. An overview of the ArcGIS settings in this study can be found in Appendix C4.

Vegetation data collected from the field experiment was analysed using Non-metric Multi-dimensional Scaling (NMDS), with a Bray-Curtis distance measure, to demonstrate the compositional (dis)similarity between the plots. An Analysis of Similarity (ANOSIM) test was used to test if there were statistical differences between the plant communities of the plots over time, between sites and between treatments. To determine which species were responsible for statistic differences between the plots, an Indicator

Species Analysis was performed. An overview of the R packages and functions used can be found in Appendix C5.

3 Results

3.1 Baseline biodiversity

3.1.1 Invertebrate diversity

A significant difference in invertebrate alpha diversity between the study areas was found ($F = 5.690$, $p = 0.00172$). Alpha diversity was highest in LP (mean 6.45, n = 11). No significant differences were found between BHP, LvO and SVV, which had a mean alpha diversity of 4.42 (n = 12), 4.86 (n = 29) and 4.83 (n = 12) respectively (Appendix D).

3.1.2 Plant diversity

In total, 51 species were observed during the vegetation recordings of September 2020. Only 13 of these were observed in BHP. Most BHP plots were mainly covered by *Lolium perenne* and *Holcus lanatus*, thus conforming with a phase 0 grassland (9 out of 12 plots). 1 plot was classified as phase 1, and 2 were phase 2. LvO had a similar species composition as BHP: 15 species were recorded and most LvO plots were dominated by *Lolium perenne* (>90% cover in 25 out of 30 plots). This resulted in only 1 phase 1 plot, 1 phase 2 plot and 28 phase 0 plots in LvO. SVV was relatively species-rich, but still dominated by *Lolium perenne*. A total of 23 species were recorded in SVV. Classification resulted in 1 plot of the phases 1, 2 and 3 each, and 9 plots of phase 0. LP was most species-rich, with a relatively high abundance of *Cardamine pratensis* and no *Lolium perenne* at all. In total, 32 species were found in LP, many of which are characteristic for (moderately) wet and nutrient-rich soils (e.g., *Phalaris arundinacea*, *Schedonorus arundinacea*). Some typical hay meadow species, such as *Phragmites australis*, *Caltha palustris* and *Hypochaeris radicata*, were observed as well. All LP plots were in between phase 3 and 4.

Significant difference in plant species number between the polders was found ($F = 20.17$, $p = 3.08e\text{-}09$). Mean plant species number per plot was 4.58 in BHP (n = 12), 8.33 in LP (n = 12), 3.77 in LvO (n = 30) and 6.58 in SVV (n = 12). Species number in LP and SVV was significantly higher than species number in BHP and LvO (Fig. 1).

With the use of the Kriging tool, the number of plant species per square meter was estimated within the defined study areas (Fig. 2). The pattern demonstrated by the map complied with the results mentioned above.

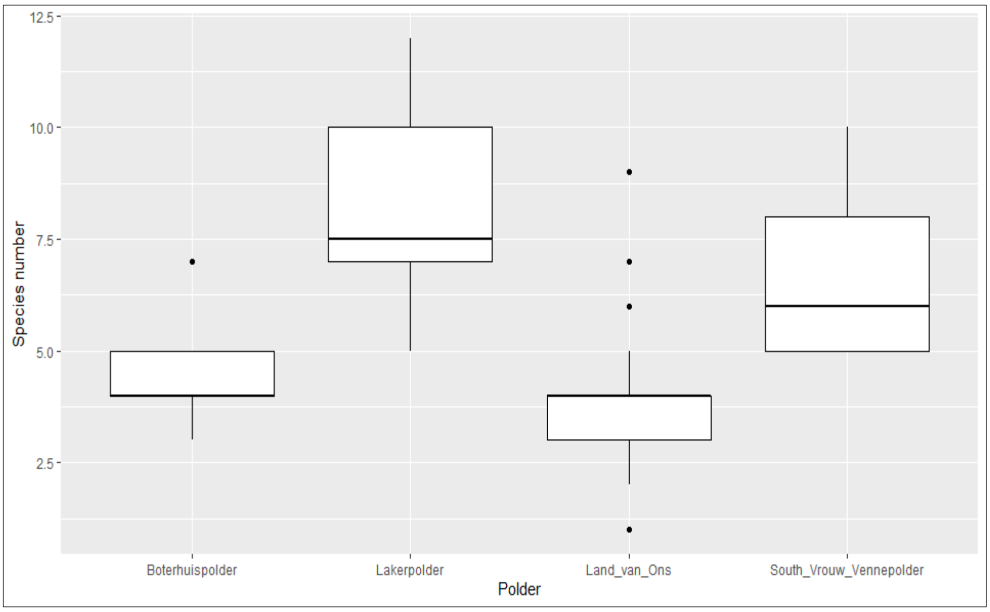

Figure 1 Number of plant species per plot in each polder.

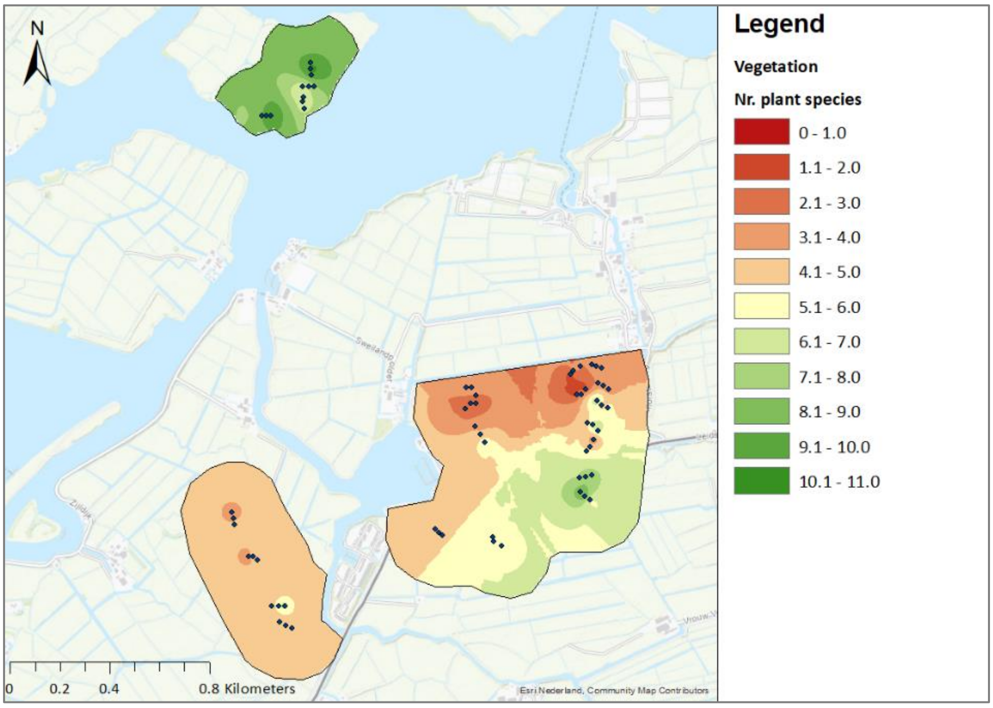

Figure 2 Estimated species number per square meter in LP (north), BHP (southwest) and VV (southeast) based on vegetation survey of September 2020.

3.2 Soil layers

Thickness of the clayey topsoil varied from 34 to >120 cm (Fig. 3). Mean thickness was 72.4 cm in BHP, 48.5 in LP, 47.2 in LvO and 67.8 in SVV. Mean depth at which intact peat started was 77.25 in BHP, 57.6 in LP, 65.7 in LvO and 76.7 in SVV (Fig. 3). No peat was retrieved up to the maximum reaching depth of the auger (120 cm) at all three sample locations in one field of SVV (shown in white in soil layer maps). Altogether, these findings resulted in the thickness of the layer that contained degraded peat, with a mean of 4.8 cm in BHP, 9.1 in LP, 18.5 in LvO and 8.8 in SVV (Appendix E).

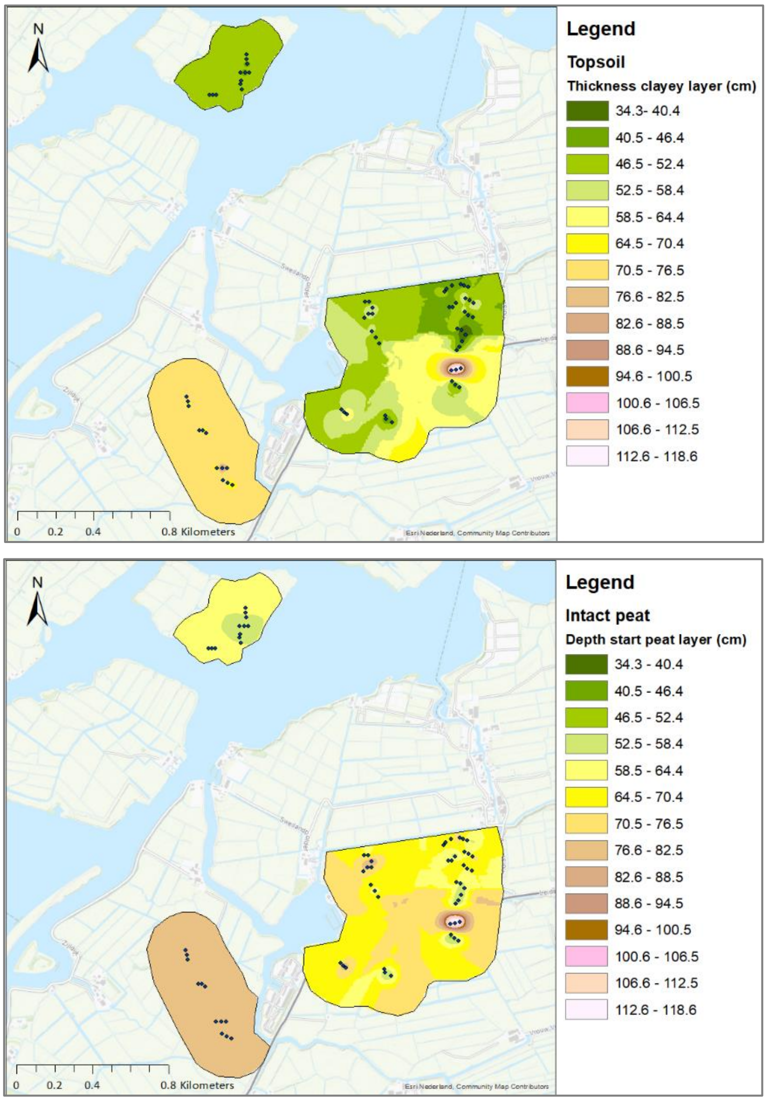

Figure 3 Top: Estimated thickness of clayey topsoil. Bottom: Estimated depth of the start of the intact peat layer.

Measures on SOM, as determined with LOI, complemented most of these findings, as spatial patterns of SOM largely match those of the distinguished layers. Lower SOM contents were found at all three depths in BHP, whereas higher SOM contents were already found at 10-20 cm in LP (Table 1, Fig. 4). In LvO, SOM varied widely at 35-45 cm (7.01-78.15%), which reflected the heterogeneity of the depths and thickness of the various soil layers.

Kriging was unable to generate detailed interpolations of SOM data of BHP at 35-45 cm and SOM data of LP at 60-70 cm, resulting in maps that could only demonstrate the mean SOM values of the designated study area.

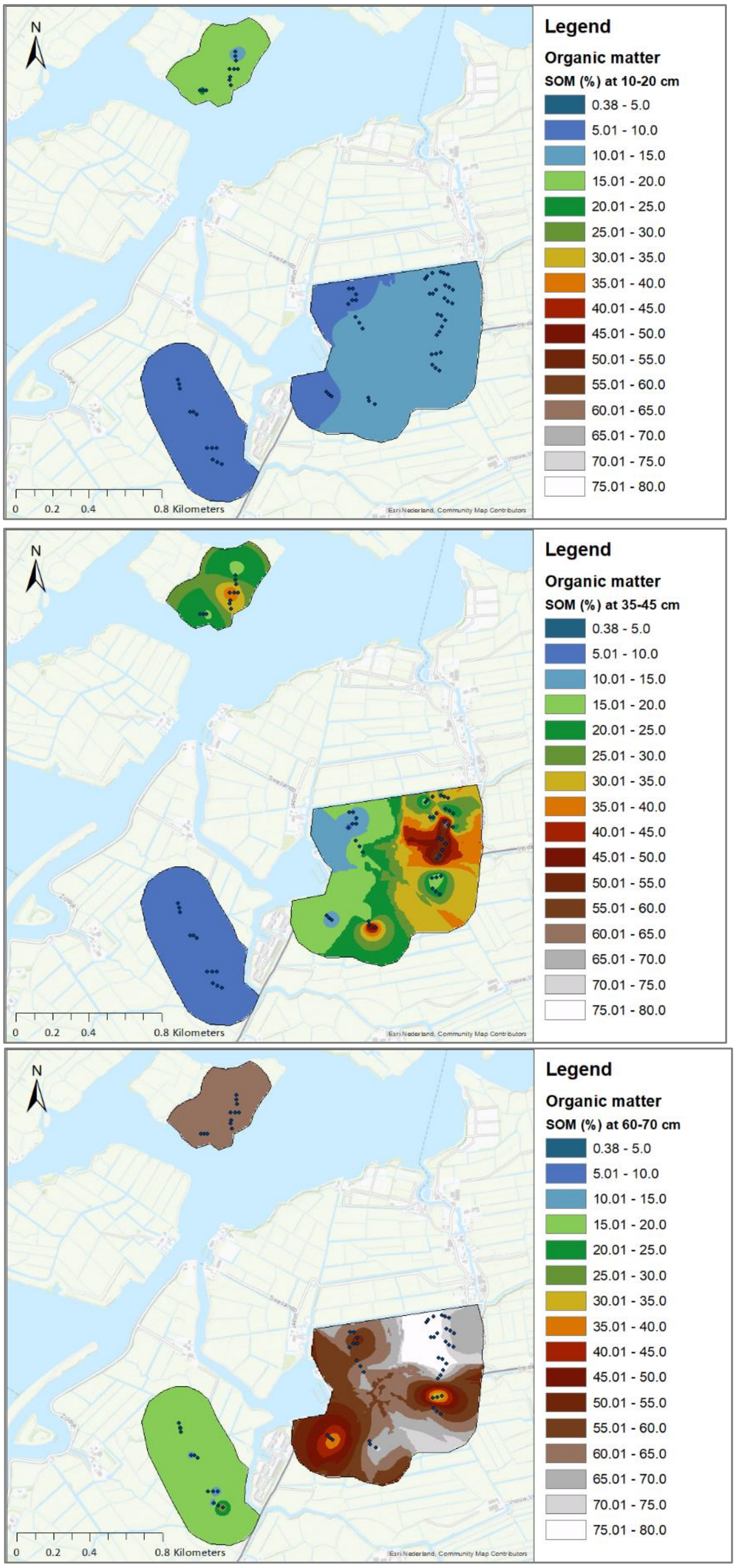

Figure 4 SOM content at a soil depth of 10-20 cm (top), 35-45 cm (middle) and 60-70 cm (bottom).

3.3 Topsoil chemistry

The C:N ratio ranged between 10 and 14. Mean C:N ratio was highest in LP and lowest in BHP (Table 1). Interpolation of C:N in LP was inadequate. Visible spatial correlation between C:N ratio and SOM was limited (Appendix F).

Spatial patterns of soil C% and N% were similar. Both soil properties were especially high in the two middle fields of LP and the eastern side of LvO, and relatively low in the two outer fields of LP and the northern part of BHP (Appendix F).

Soil pH was remarkably high in the three middle plots in the north-western part of LvO (6.7-7.3; Fig. 5). A relatively low pH was found at all other locations (4.8-6.4). Mean soil pH was lowest in BHP (Table 1).

P mg L^{-1} was lowest in LP and the north-eastern fields of LvO (Fig. 6). Highest levels of P (in both mg L^{-1} and mg kg^{-1}) were found in LvO as well. The mg kg^{-1} data showed higher variability than the mg L^{-1} data.

Overall, a clear dividing line between the western and eastern side of LvO was visible in the maps of soil chemistry, and in LP a distinction between the middle and outer fields could be made. Further spatial correlation was minimal.

Table 1 Mean values of soil properties for each study area. Standard deviations (±SD) are given in brackets.

	BHP	LP	LvO	SVV
SOM 10-20	7.25 (± 1.88)	17.33 (± 6.62)	11.55 (± 2.74)	12.26 (± 3.48)
SOM 35-45	8.78 (± 2.12)	29.13 (± 10.02)	30.35 (± 19.80)	21.5 (± 15.96)
SOM 60-70	17.89 (± 9.32)	63.14 (± 15.36)	70.84 (± 15.09)	46.21 (± 32.15)
Moisture 10-20	25.49 (± 3.01)	50.1 (± 6.73)	32.91 (± 2.65)	31.41 (± 4.17)
Moisture 35-45	32.67 (± 5.30)	64.34 (± 8.74)	48.9 (± 13.85)	39.68 (± 9.29)
Moisture 60-70	51.39 (± 9.91)	81.11 (± 5.69)	75.34 (± 6.68)	59.42 (± 18.38)
C:N	10.97 (± 0.62)	12.4 (± 0.74)	11.53 (± 0.86)	
Soil N%	0.5 (± 0.08)	0.63 (± 0.19)	0.63 (± 0.16)	
Soil C%	5.51 (± 1.05)	7.83 (± 2.33)	7.26 (± 1.70)	
pH	5.08 (± 0.18)	5.38 (± 0.28)	5.55 (± 0.57)	
mg P L^{-1}	0.38 (± 0.10)	0.33 (± 0.07)	0.42 (± 0.10)	
mg P kg^{-1}	3.73 (± 0.10)	3.29 (± 0.73)	4.18 (± 1.03)	

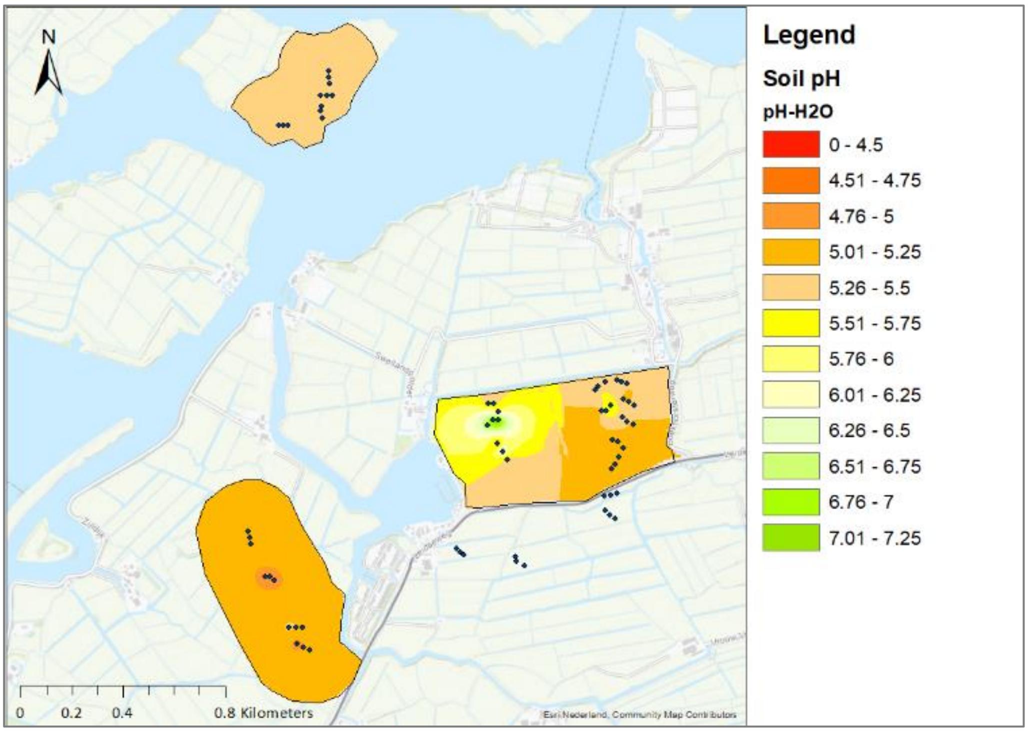

Figure 5 Soil pH in LP, BHP and LvO.

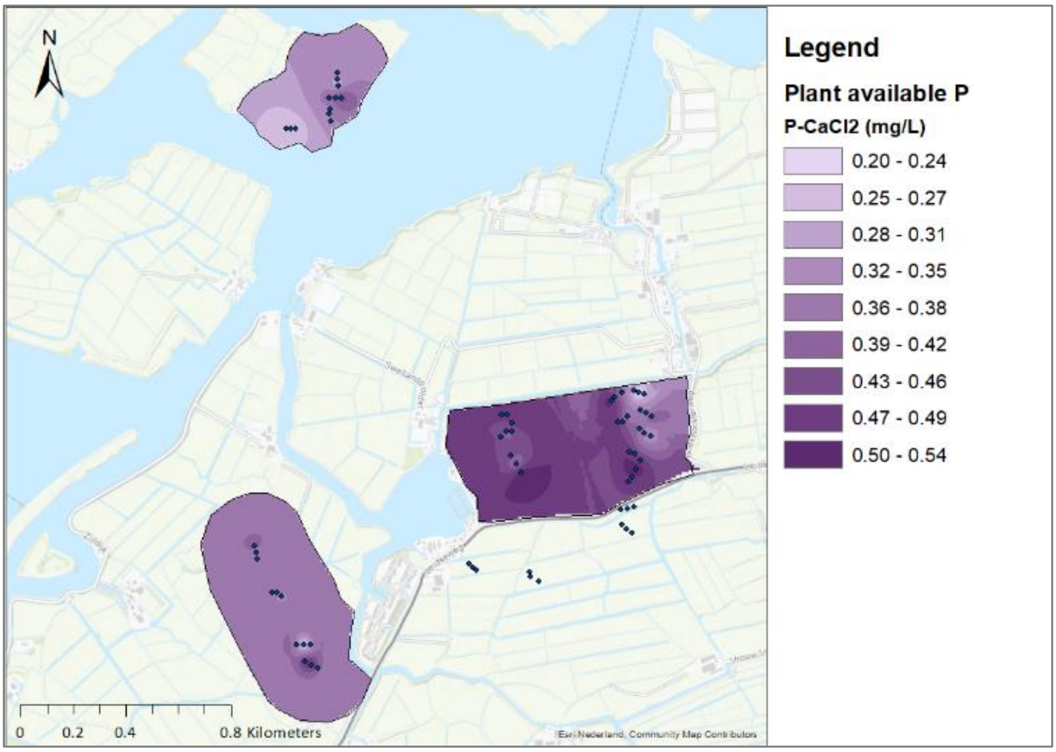

Figure 6 Plant available P in LP, BHP and LvO.

3.4 Groundwater

Water levels were kept at approximately 35-75 cm below ground level in LvO and SVV (Fig. 7). Because of differences in elevation, water levels in the centre of the BHP study area were 60-75 cm below ground level, but only 30 cm in the outer fields. The difference between water level and ground level was only 25 cm in most of LP. Moisture content ranged from 20.8-38.7% at 10-20 cm in BHP, LvO and SVV. In LP moisture content was remarkably higher at 10-20 cm depth (36.69-65.51%), and in most of the other layers as well. At all three depths, moisture content of the soil samples was lowest in BHP. High variability was observed in LvO at 35-45 cm (24.32-72.07%).

Spatial patterns of the estimated groundwater levels were not reflected in the moisture content maps. In LvO, spatial patterns between SOM and moisture content appeared to be similar to some extent, especially at the two deeper layers.

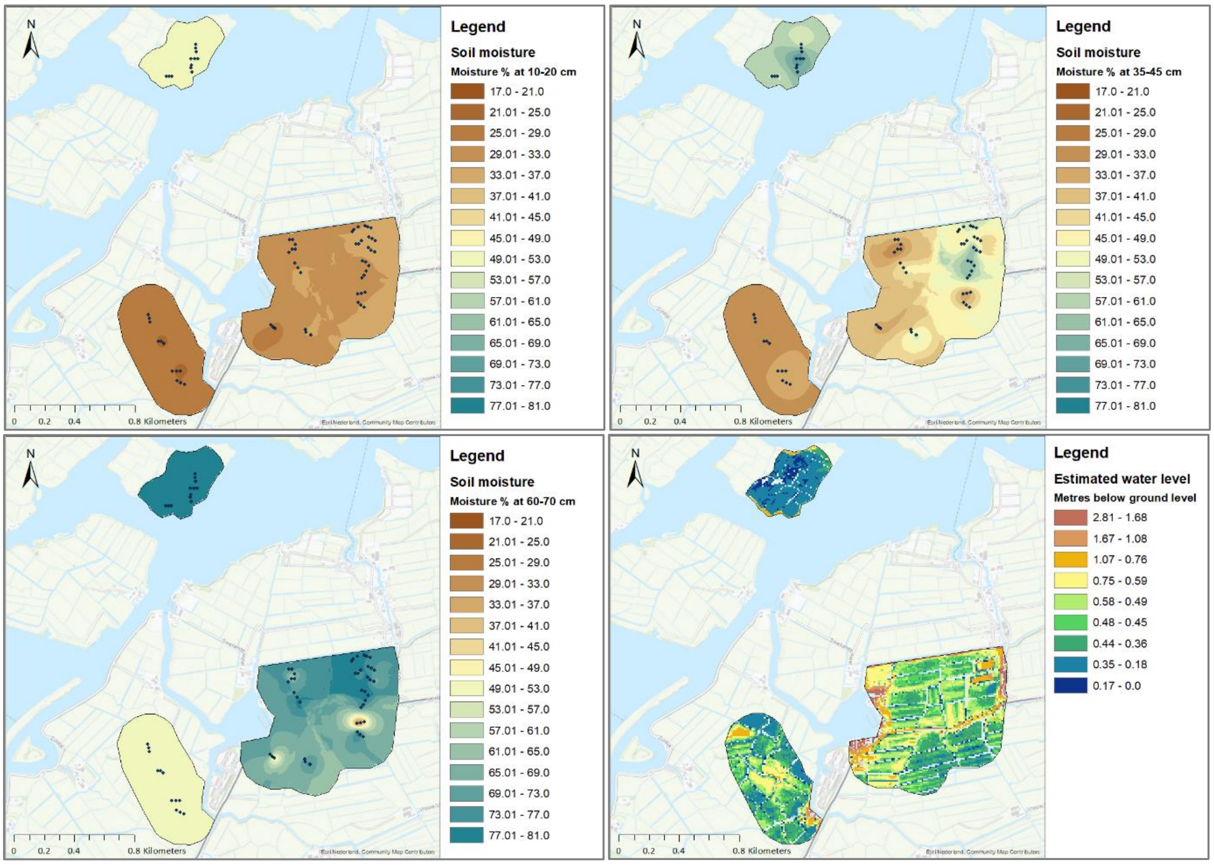

Figure 7 Soil moisture content at a depth of 10-20 cm (top left), 35-45 cm (top right) and 60-70 cm (bottom left). Bottom right figure demonstrates the estimated difference between ground level and groundwater.

3.5 Field experiment

During the initial observations (t0), BHP-1 had a mean of 4.25 species per plot, BHP-2 had 5.83 and LvO-3 had 4.58. During the final observations (t2), a mean of 5.75 plant species per plot was found in BHP-1, 7.08 in BHP-2 and 3.75 in LvO-3.

The ANOSIM of the NDMS demonstrated a significant difference in species composition of the plots between t0 and t2 (stress = 0.225, R = 0.084, p = 0.003; Table 2). The stress value, greater than 0.2, indicates that the data is no represented very well. The relatively low R value suggests an even distribution of high and low ranks within and between groups. The species responsible for the differences between t0 and t2 were mainly grasses (*Lolium perenne*, *Alopecurus pratensis*, *Poa annua*, *Bromus hordeaceus*, *Phalaris arundinacea* and *Agrostis sp.*), as well as common dicots: *Cerastium fontanum*, *Trifolium repens* and *Bellis perenne*.

Differences in species composition of the plots between the three sites were found at both t0 (stress = 0.173, R = 0.301, p = 0.001) and at t2 (stress = 0.191, R = 0.408, p = 0.001). At t0, the plots of BHP-2 had a significantly lower abundance of *Lolium perenne* and *Poa annua* than the plots of the other sites (p = 0.005), and a higher abundance of *Bromus hordeaceus*, *Bellis perenne* and *Ranunculus repens* (p = 0.005). Abundances of *Cerastium fontanum* and *Taraxacum officinale* were significantly higher in LvO-3 plots (p = 0.015).

At t2, *Lolium perenne*, *Ranunculus repens*, *Phalaris arundinacea*, *Poa annua*, *Agrostis sp.*, and *Rumex conglomerata* were responsible ($p < 0.05$) for the differences in species composition of the three sites. Especially *Lolium perenne* abundance was low in both BHP-1 and BHP-2 plots compared to LvO-3 plots (p = 0.005), and the abundance of *Trifolium repens* and *Ranunculus repens* were significantly higher in BHP-1 and BHP-2 (p = 0.015).

The only sown hay meadow species that were observed in the experimental plots were *Cerastium fontanum* and *Trifolium pratense*, both in low abundance at t2.

At the end of the experiment, no significant differences in species composition between the four treatments were found (stress = 0.191, R = 0.052, p = 0.090). Visualisation of the NMDS mainly demonstrated clustering based on location and moment of recording rather than treatment (Fig. 8).

Table 2 Summed species cover of all plots at each site at t0 and t2. Species that were present in seed mixture are in bold.

	BHP-1		**BHP-2**		**LvO-3**	
	t0	t2	t0	t2	t0	t2
Agrostis sp.	0	355	0	26	0	3
Alopecurus geniculatus	0	10	0	0	0	0
Alopecurus pratensis	11	0	36	0	0	1
Anthoxanthum odoratum	0	0	36	0	0	0
Bellis perenne	2	1	43	1	0	0
Bromus hordeaceus	1	0	61	0	5	0
Capsella bursa-pastoris	0	0	0	0	1	0
Cardamine pratensis	0	0	0	2	0	0
Cerastium fontanum	0	1	8	4	40	0
Ficaria verna	0	0	0	0	10	0
Unidentified grass	0	0	5	0	0	0
Holcus lanatus	0	271	370	330	245	260
Juncus sp.	0	0	0	47	0	0
Lolium perenne	985	340	445	380	795	935
Persicaria sp.	0	1	0	0	0	5
Phalaris arundinacea	0	0	30	222	0	0
Plantago sp.	1	7	0	1	0	0
Poa annua	190	55	0	12	71	5
Poa trivialis	0	0	0	0	20	0
Potentilla anserina	0	0	1	11	0	0
Ranunculus repens	16	108	170	135	1	6
Rorippa sp.	0	0	0	0	0	1
Rumex acetosa	0	0	1	2	2	6
Rumex conglomerata	0	0	0	5	0	0
Rumex crispus	0	1	1	0	0	0
Scorzoneroides autumnalis	0	1	0	0	0	0
Symphytum officinale	0	0	0	0	1	0
Taraxacum officinale	2	0	3	7	27	3
Trifolium pratense	0	1	0	2	0	0
Trifolium repens	19	71	12	45	1	2
Vicia sp.	0	0	0	0	0	1

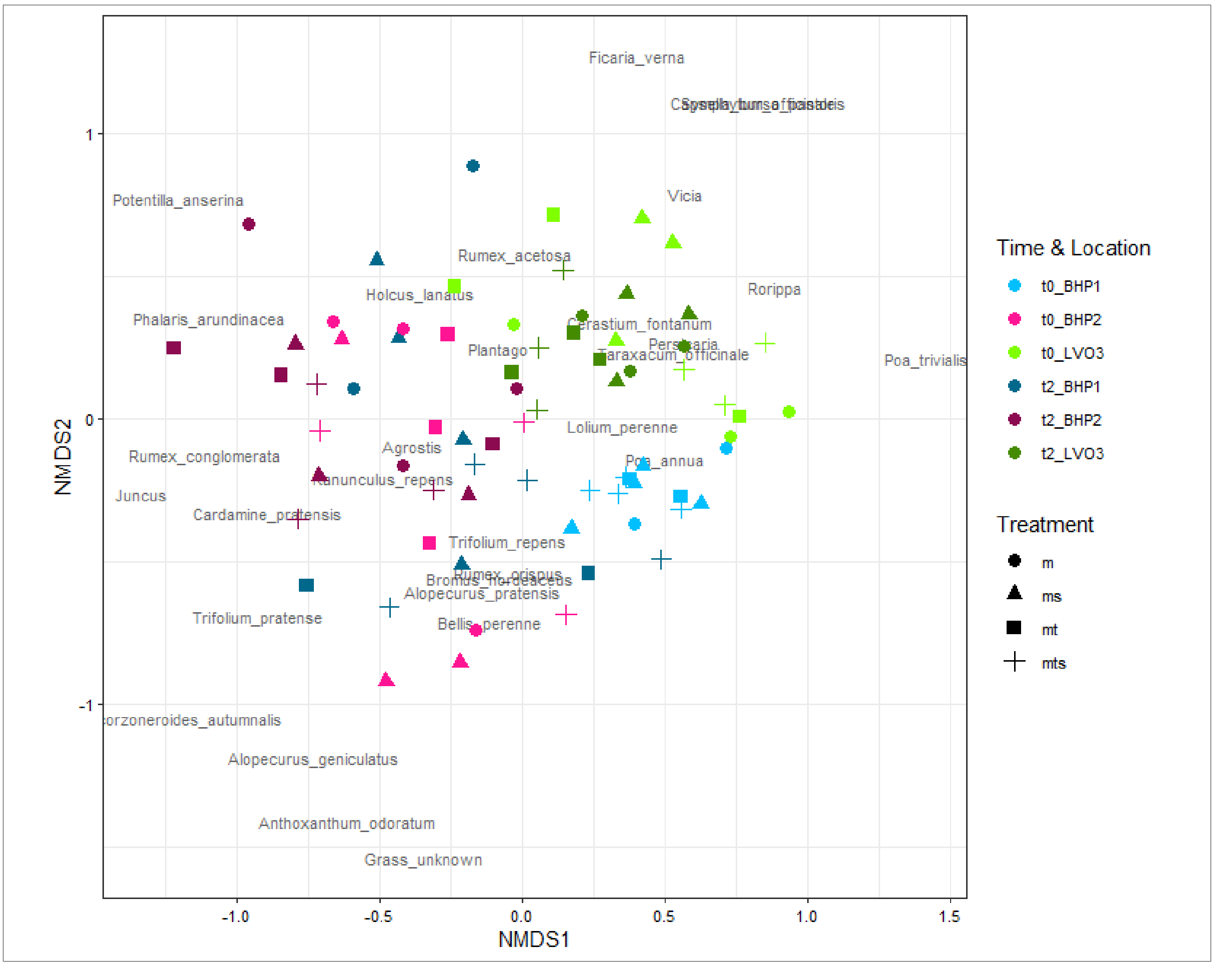

Figure 8 NMDS plot of the plant communities before and after the field experiment. The colours demonstrate at which moment, and in which field the vegetation has been observed. Shapes indicate the treatments applied after the observation at t0.

4 Discussion

This study set out to determine the most effective strategies to facilitate a controlled regime shift from the severely degraded grasslands to species-rich hay meadows in the peatland area. The findings of this study form the foundation for some integrated recommendations regarding the envisaged regime shift. Using LP as a reference area, the possibilities for a species-rich hay meadow system in the target area (LvO) are explored below. Suggestions for management options to change biodiversity here are based on an overview of the baseline situation (plant and invertebrate biodiversity) and the underlying soil properties (type, chemistry and moisture). Spatial patterns between these variables were sought to identify potential relations between the state of an ecosystem and the underlying EP.

Overall, the results of this study show spatial correlation between plant species number and soil properties such as SOM, moisture content, C% and N%. However, some analyses resulted in inconclusive patterns and more sampling will be required to resolve this. The field experiment should have provided insight in the effectiveness of management practices for the establishment of hay meadow species. The outcome indicated that continuation of the experiment is needed to improve results and the understanding of the effects of management.

Some of the results may have been affected by the limitations of this research. These limitations should be considered before the results can be interpreted and translated into management recommendations. Therefore, the limitations will be discussed below first. Subsequently, the findings of this research will be interpreted and linked to existing literature, after which the final recommendations for further action will be given.

4.1 Limitations of this study

The vegetation baseline assessment was executed in September 2020. Fertilization has been ceased after this assessment and based on the appearance of the meadow in spring 2021, botanical diversity seems to have increased already. However, this could not be concluded from an additional field survey done in the same study area in May 2021, in which the plant species composition in ditches and ditch banks were re-analysed (Bos & Van Duijn, 2021). Based on this information, it seems likely that there are no significant differences in species richness in the fields either, so it can be assumed that the September 2020 data is still relevant for this study.

There were some clear limitations to the soil analyses of this study. First of all, visual analysis of soil layers was likely not very accurate, mainly because of a blurry transition between soil layers. This may have led to some incorrect estimates regarding the thickness and depth of the layers. However, in most cases the SOM data can be used to validate the findings of this study, and these will play a central role in the further integration of the results. Secondly, the soil analyses may have been affected by the date of sampling. Since the samples have been taken over a period of multiple weeks, weather and storage conditions may have affected soil moisture content. However, the spatial patterns of soil moisture broadly match those of SOM and groundwater levels. Therefore, the effect of the mentioned variables on the results will likely not be significant. Thirdly, the predetermined sample locations may have had major implications for the Kriging analysis, which uses distance between points for interpolation. The distribution of sampling points may be the cause of the apparent east/west pattern in the soil maps. To improve the accuracy of this analysis, it would be sensible to select more evenly distributed sample locations throughout the study area. Additionally, it should be kept in mind that the scale used in this study's Kriging analysis might not cover the potential heterogeneity of soil properties at smaller scales. The failure of Kriging for some of the soil properties may be caused by one of these last two limitations. Despite the potential inaccuracies, the results of the soil assessment provide an overview of the baseline situation in typical livestock meadows in the Dutch peatland area. The outcomes may serve as a useful starting point for further work on regime shifts in the peatland agroecosystem.

It is obvious that the estimation of the groundwater levels, based on measurements on the main water system, cannot be truly accurate, because soil properties and hydrological processes are neglected. It is understandable that, considering this shortcoming and the ones regarding moisture samples and Kriging, the patterns of the groundwater maps do not match at the smaller scale. However, combining the results undoubtedly provides a number of important insights on which a first set of conclusions can be based.

The last major limitation of this study is the use of LP as reference area for the species-rich hay meadow system. Based on the data analysis in this study, it seems that LP does not fully resemble a (high quality) species-rich hay meadow, which implies that some of the EP are not completely suitable. There may be some limiting factors that hinder the development of a hay meadow system, such as low soil pH, high soil salinity and potentially low species introduction (because LP is an island and there are few neighbouring communities). However, there are no specific target values for all soil properties. Therefore, LP currently is the best reference system available to determine target values for soil properties.

4.2 Contrasting baseline biodiversity patterns between polders

Of all areas, LP had the most biodiverse ecosystem in terms of invertebrate diversity and plant species number. The LP plant species composition demonstrated some resemblance to a species-rich hay meadow. This suggests that the EP and management regime in this area are, to a certain extent, favourable, and that LP can be used as reference system for species-rich hay meadows in this area. The plant communities of BHP, LvO and SVV complied with a phase 0 grassland, indicating adverse conditions for hay meadow species. These findings can be associated with the differences in EP found between the polders, as well as with the differences in the responsible management between LP and the typical livestock meadows.

4.3 Underlying soil properties

4.3.1 Soil type based on SOM

The characterisation of the soil type is important to determine which are the most suitable species to introduce into the system. Three soil layers were distinguished visually: the clayey topsoil, the layer of degraded peat and the intact peat layer. SOM data can be used to improve the accuracy of this evaluation. There seems to be no general consensus on the SOM content of peat, but in compliance with the Dutch soil directive, this study will consider soils with a SOM content of 15-100% to be peat soils.

The visual evaluation of soil layers in BHP suggested a clayey layer of approximately 60-80 cm. This finding was confirmed by the low SOM content in most of the soil samples of all three depths. The occurrence of non-degraded organic material beyond 70 cm suggests the existence of a peaty layer beneath the clay, but this could not be proven without further analysis. For LP, results of the visual analysis suggested a clayey topsoil of 48.5 cm on average. However, LOI resulted in an average SOM content >15% at the depth of 10-20 cm, and higher SOM content in the 35-45 cm layer. This implies that the visual evaluation may have resulted in an overestimation of the thickness of a clayey topsoil. It is probable that degraded peat has been regarded as clayey topsoil at some locations. The visual and chemical analyses demonstrate a top layer of clay in VV. Below the 10-20 cm layer, SOM content suggests a highly variable soil profile. The maps show a thicker clayey layer in the west of LvO, and a high SOM peat layer in the rest of VV.

These findings suggest that land managers may choose to facilitate for either a clay- or peat-based plant community. In the latter case, removal of the clay topsoil will enable the development of vegetation that favours a peaty substrate.

4.3.2 Diverse topsoil chemistry

No spatial correlation was found between C:N ratio and plant species richness. Deru et al. (2012) found a range of 10.3-14.1, with no significant differences in C:N ratio between agricultural and natural grasslands on peat (Deru et al., 2012). These results are in line with the findings of this study. Therefore, it seems unnecessary to include this soil property in the management strategy.

No spatial correlation was found between pH and plant species richness, but there are some recommendations regarding peat soil pH available. Generally, a pH of 5.5 or higher is considered to be suitable for species-rich hay meadows (BIJ12, 2017). This suggests that the soils of LP, BHP and the south-eastern part of LvO might be too acidic for hay meadow species. However, alteration of soil pH can be problematic, because it affects nutrient availability (Van Eekeren et al., 2019), and since the acidic soils of LP support some hay meadow species such as *Caltha palustris*, changing the soil pH in the target area does not have to be prioritized.

To a certain extent, high plant diversity corresponded to lower values of P. Soil P values found in most of LvO (mean 4.18 mg kg^{-1}) seem to be relatively high. A study by Ehlert et al. (2007) estimated a mean

P content of only 2.7 mg kg^{-1} for arable land on peat and 3.7 mg kg^{-1} on clayey peat. Moreover, according to Dutch phosphate standards, grassland soil P content of >3.4 mg kg^{-1} is considered high. Therefore, it seems desirable to reduce soil P in most of LvO. To estimate the time and effort required for P reduction through mowing and swath removal, more insight in the total soil P pool is required. However, the effectiveness of this method on peat soils is doubted, so the removal of the topsoil might be essential (Van Mullekom et al., 2014). This too requires more insight into the soil P pool, particularly into the thickness of the nutrient-rich layer that would have to be excavated.

4.3.3 Higher groundwater levels required

Although the limitations of the analyses indicate the inaccuracy of the results, it is clear that there is a difference in groundwater levels and soil moisture contents between the polders. In LP, highest plant diversity does not correspond to highest soil moisture content. However, moisture content is considerably lower in LvO. For both the development of hay meadow vegetation and the prevention of further peat degradation rewetting would be desired.

Unfortunately, rewetting cannot be done without considerable risks. It is well known that soil moisture content affects the availability of N and P (Araya et al., 2013; Van De Riet et al., 2013), which implies that rewetting might have adverse effects on the development of a species-rich hay meadow. The complete extent of this effect cannot be estimated from the conducted analyses, since only plant available P (P-$CaCl_2$) has been measured. The results already demonstrated high P availability and there might be even more immobile P in the LvO soil that could be released when rewetted (Van De Riet et al., 2013; Van Eekeren et al., 2019). It is clear that thorough removal of nutrients is required for the creation of a species-rich hay meadow.

4.4 Recommendations based on initial assessments

To initiate the regime shift in the target area, or other typical livestock meadows in the Dutch peatland area, different strategies can be applied. Essential elements of such strategies should be the interruption of the *Lolium perenne* dominance and the reduction of soil nutrient concentrations. Both can be achieved through a strict long-term mowing regime. Quicker results can be achieved through topsoil removal, but this is a highly invasive and costly measure.

The timing of mowing will affect competition within the plant community and with that the ratio between forbs and grasses (Schippers et al., 2019). A common mowing regime consists of a first cut in May – before seed production – and states that fertilizers should not be applied afterwards to prevent regrowth (Boob et al., 2019; Schippers et al., 2019). Mowing again in late summer is highly recommended (Schippers et al., 2019). As mentioned in various studies, swath should always be removed after mowing to take out nutrients from the system, creating a less favourable environment for the fast-growing grasses (Bakker, 1989; Myklestad, 2004; Pavlů et al., 2021; Tallowin & Smith, 2001). To speed up the P removal through mowing, grassland production can be increased by adding *Trifolium* into the grass mix (Van Mullekom et al., 2014). Nevertheless, the development of a species-rich hay meadow will take at least four years with the use of these mowing strategies (Schippers et al., 2019). Van Mullekom et al. (2014) suggest that it might even take up to over 40 years until P concentrations in the upper soil layers are suitable.

Topsoil removal has been considered a successful measure in several grassland restoration studies (Klimkowska et al., 2007; Losvik & Austad, 2002; Mandigers et al., 2016). In the target area, it will immediately break the grass dominance and reduce nutrient concentrations. Moreover, removal of the clayey layer will expose the peat layer beneath it. After topsoil removal, rewetting and introduction of the desired species will be essential to prevent the development of undesired vegetation and facilitate the development of the species-rich hay meadow system (Losvik & Austad, 2002; Pavlů et al., 2021; Van der Hoek, 2005; Van Mullekom et al., 2014). To determine the required excavation depth, more insight in plant available P and the total P stock is required.

4.5 Inadequate management in current environment

The results of the field experiment were inconclusive, indicating that the management treatments may have been ineffective. The failed species introduction may also be due to the prevailing unsuitability of the EP in the experimental sites. In addition, the lack of conclusive results may have been caused by limitations regarding the experimental set-up, such as the duration of the experiment or the timing of mowing and sowing. A multiannual experiment, consisting of more cuts and sowing in autumn, could have led to establishment of sown species (P. De Groot, personal communication, 23 March 2021). However, this does not preclude the ineffectiveness of the treatments, which is discussed below.

4.5.1 General trends

Overall, some trends could be observed from the field experiment. For instance, it is clear that the *Lolium perenne* cover in BHP-1 has strongly reduced over time, whereas an increase can be observed in LvO-3. However, the low R value, resulting from the NDMS comparing the species composition of t0 and t2, suggests an even distribution of high and low ranks within and between groups, indicating that the overall community structure has not changed much. It seems that, generally, the dominant grasses have been replaced by other grass species.

4.5.2 Causes

At the start of the experiment, there was no difference in species composition between the plots of the treatment groups, so it can be assumed that the starting point was equal for the plots within the same site. However, there was no difference in species composition at the end of the experiment either. Most likely, the treatments have failed in breaking the grass domination, which renders seedlings unable to overcome the competition for light. This, combined with the unfavourable soil conditions and potential limitations of the experimental set-up, may have hindered the establishment of (sown) hay meadow species. Even though, the true cause of this failure cannot be identified with certainty, it is clear that more effort is required to create a suitable environment for the initial species introduction. Continuation of this experiment could improve the results, since repeated mowing and tillage could reduce the grass density and enhance the chance of establishment and survival of seedlings.

4.5.3 Future prospects for species introduction

Once environmental circumstances are more suitable, the introduction of hay meadow species should be more successful. Natural colonization however seems unlikely since there are few nearby species pools from which seed dispersal is possible. Therefore, the development of hay meadow vegetation is dependent on the sowing of locally sourced seed mixtures (Mitchley et al., 2012). It might be beneficial to adjust the composition of these mixtures based on the soil type, nutrient concentration and groundwater level of the target area. This means that in earlier phases, species such as *Trifolium pratense*, *Plantago lanceolata* and *Cerastium fontanum* could already be introduced on dry nutrient-rich peat soil to create a species-rich grassland. Once soils are wet and impoverished, the introduction of *Caltha palustris* and *Dactylorhiza praetermissa* might be effective (Schippers et al., 2019).

5 Conclusions

This study demonstrated that profound knowledge of soil properties is essential to determine what measures are needed for the initiation of a regime shift in the agricultural peatlands. The prevailing EP in the typical livestock meadows of LvO and BHP, appeared to be unsuitable for the establishment of the species-rich hay meadow system. The results indicate that drastic measures are required to facilitate the regime shift towards the desired agroecosystem and reduce the environmental problems in target area.

Both a strict mowing regime and topsoil removal will be useful in creating the required environmental conditions, but the duration and costs of these measures will differ significantly. Rewetting is desirable, but water levels should not be elevated before soil nutrient concentrations are sufficiently low. Natural introduction of hay meadow species is unlikely, therefore sowing will be essential. Further research is required to determine the details regarding the implementation of the suggested measures.

Eventually, a heterogenous agricultural landscape, including species-rich hay meadows, should be developed to reduce the typical environmental issues in the peatland area. Based on the approach used in this study, a general workflow can be set up to determine the strategies required to facilitate a wide array of regime shifts to species-rich agroecosystems. The lessons drawn from this study and potential follow-up studies can be used in similar agroecosystem restoration projects in the Dutch peatland area and other peatlands in northwest Europe.

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Conflict of interest

The author declares no conflict of interest. This research was set up to advise the Land van Ons cooperation, but this cooperation had no role in the design of this study; in the collection, analyses, or interpretation of data or in the writing of this report.

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Appendices

Appendix A: Indicator species “Vochtig hooiland”

List consists of plants, birds and insects (Lepidoptera & Orthoptera)

1 *Anacamptis morio*
2 *Blysmus compressus*
3 *Boloria selene*
4 *Briza media*
5 *Bromus racemosus*
6 *Calidris pugnax*
7 *Caltha palustris subsp. palustris*
8 *Carex aquatilis*
9 *Carex pallescens*
10 *Carterocephalus palaemon*
11 *Chorthippus montanus*
12 *Cirsium oleraceum*
13 *Colchicum autumnale*
14 *Crepis paludosa*
15 *Crex crex*
16 *Dactylorhiza incarnata*
17 *Dactylorhiza maculata*
18 *Dactylorhiza majalis subsp. majalis*
19 *Dactylorhiza majalis subsp. praetermissa*
20 *Fritillaria meleagris*
21 *Galium boreale*
22 *Gallinago gallinago*
23 *Genista tinctoria*
24 *Geranium pratense*
25 *Hypericum tetrapterum*
26 *Jacobaea aquatica*
27 *Juncus filiformis*
28 *Juncus gerardii*
29 *Limosa limosa*
30 *Lycaena tityrus*
31 *Mentha pulegium*
32 *Motacilla flava*
33 *Myosotis scorpioides subsp. nemorosa*
34 *Odontites vernus subsp. serotinus*
35 *Ophioglossum vulgatum*
36 *Pedicularis palustris*
37 *Persicaria bistorta*
38 *Phengaris nausithous*
39 *Phengaris teleius*
40 *Phyteuma spicatum subsp. nigrum*
41 *Platanthera bifolia*
42 *Pyrgus malvae*
43 *Ranunculus auricomus*
44 *Ranunculus hederaceus*
45 *Sanguisorba officinalis*
46 *Scirpus sylvaticus*
47 *Selinum carvifolia*
48 *Silaum silaus*
49 *Stethophyma grossum*
50 *Tringa totanus*
51 *Valeriana dioica*
52 *Viola persicifolia*

53

Appendix B: Study area

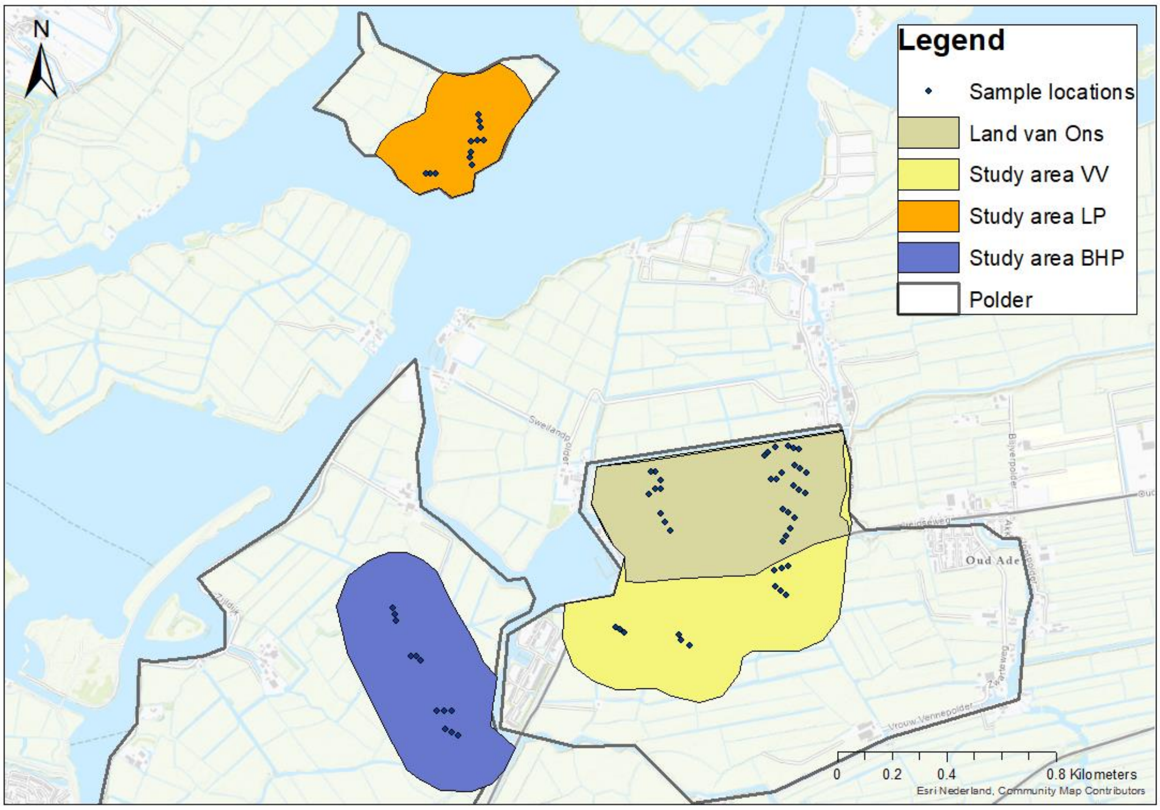


Figure B.1 Study areas for Kriging analysis based on a 200 m buffer area around the sample locations.

Appendix C: Extensive Methodologies

C1: Grassland phases

- Phase 0: >50% *Lolium perenne*
- Phase 1: <50% *Lolium perenne*, >10% other grasses (i.e., *Agrostis stolonifera*, *Poa annua*)
- Phase 2: >50% *Holcus lanatus* or *Alopecurus pratensis*
- Phase 3: >50% forbs, <50% common meadow grasses (i.e., *Lolium perenne, Holcus lanatus, Poa annua., Alopecurus pratensis*)

C2: Experimental set-up

Figure C.1 Top right: BHP-2: 52.1851, 4.5394
Bottom left: BHP-1: 52.1839, 4.5383

Figure C.2 LvO-3: 52.1918, 4.5549

Orange = MT+ (mown, tilled and sown)
Green = M+ (mown and sown)
Yellow = MT- (mown and tilled)
Blue = M- (mown)

C3: Seed mixture

- *Achillea millefolium*
- *Carex disticha*
- *Carex otrubea*
- *Centaurea jacea*
- *Cerastium fontanum subsp. vulgare*
- *Lotus corniculatus*

- *Rhinanthus angustifolius*
- *Silene flos-cuculi*
- *Trifolium dubium*
- *Trifolium pratense*
- (Potentially *Carex panicea*)

C4: ArcGIS Kriging variables

- Geographic Coordinate System: RD_New
- Study area: drawn polygon based on a 200 metre buffer area around coordinates of sample locations in each of the polders.
- Processing extent: polygon study area of the particular polder analysed
- Raster analysis – mask: polygon study area of the particular polder analysed
- Kriging specifics:
    - Kriging method: ‘ordinary’
    - Model: exponential semivariogram
    - Raster cell size: 10 metres
    - Input: coordinates of the particular polder analysed, joined with the EP data of those coordinates
    - Z-value: joined EP data

C5: RStudio variables

- Package: vegan
    - Command: metaMDS
        - K = 2
        - Distance = “bray”
    - Command: anosim
        - Distance = “bray”
- Package: indicspecies
    - Command: multipatt
        - Func = “r.g”

Appendix D: Invertebrate diversity

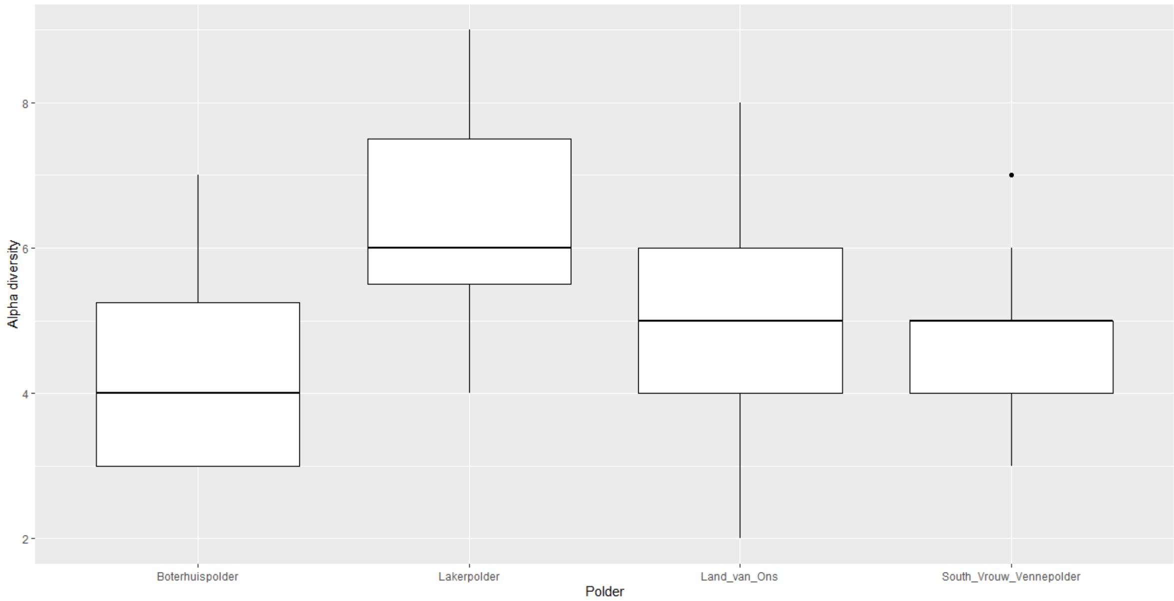


Figure D.1 Significant difference in species richness between LP & BHP (ANOVA, p = 0.0016), between LP & LvO (p = 0.0041), and between LP and SVV (p = 0.0165). Differences between the other polders were not significant (p > 0.1).

Appendix E: Degraded peat layer

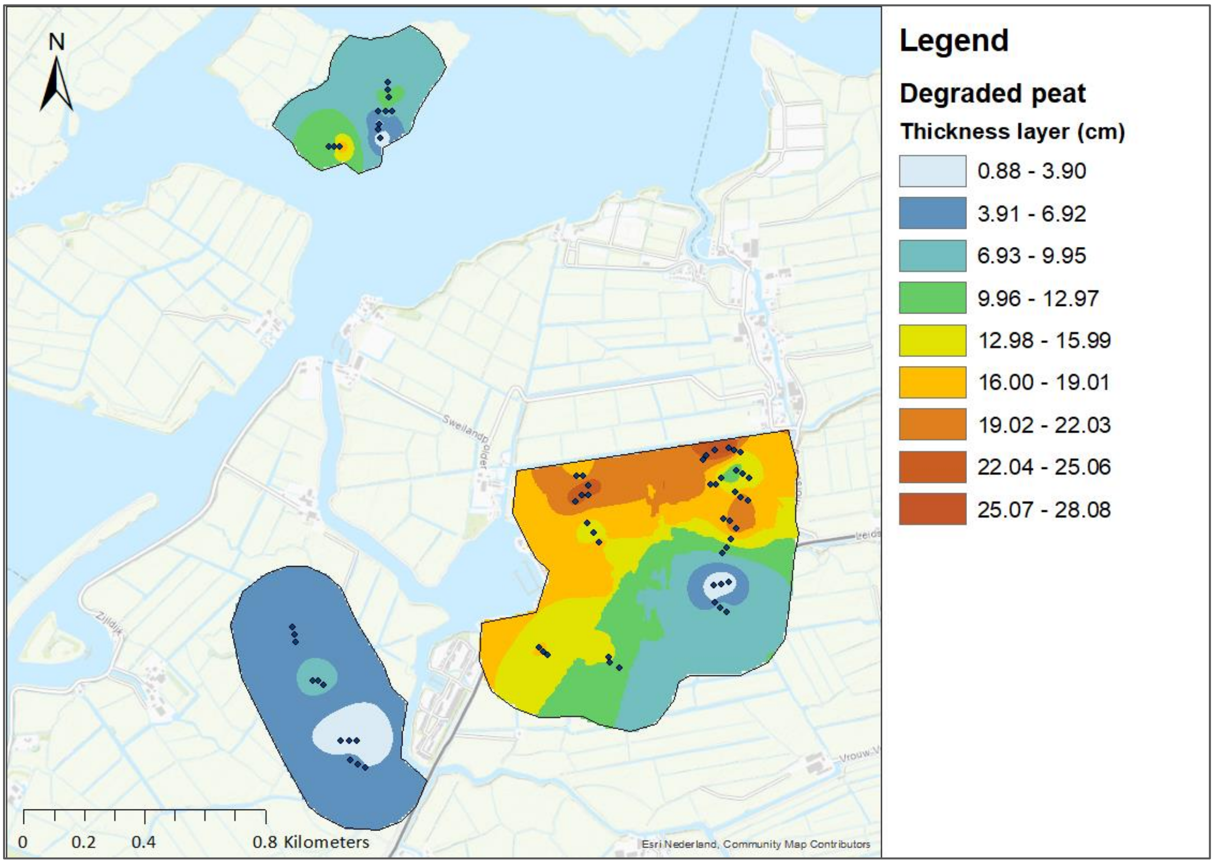


Figure E.1 Estimated thickness of the degraded peat layer in between the clayey topsoil and the beginning of the intact peat layer.

135 # Appendix F: Soil Carbon & Nitrogen

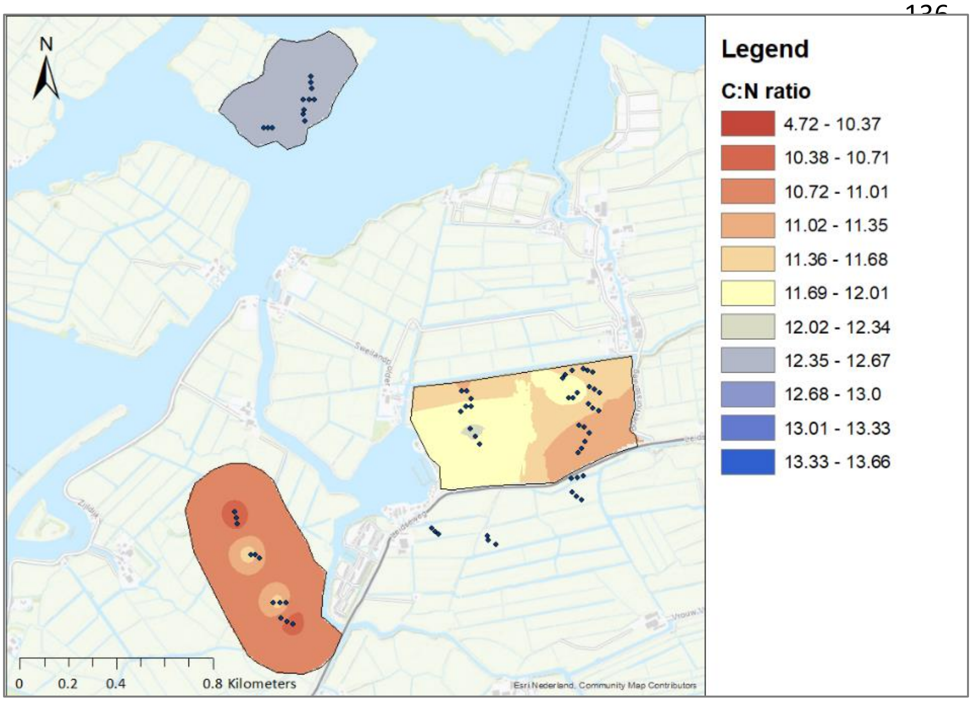
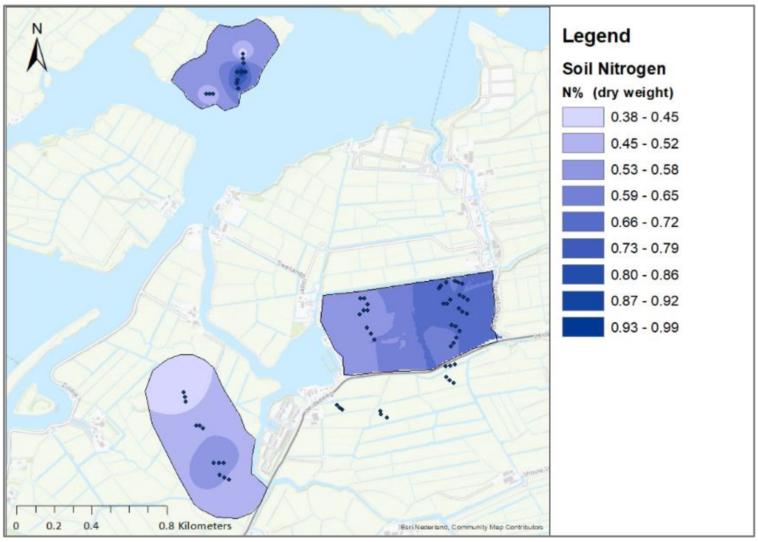
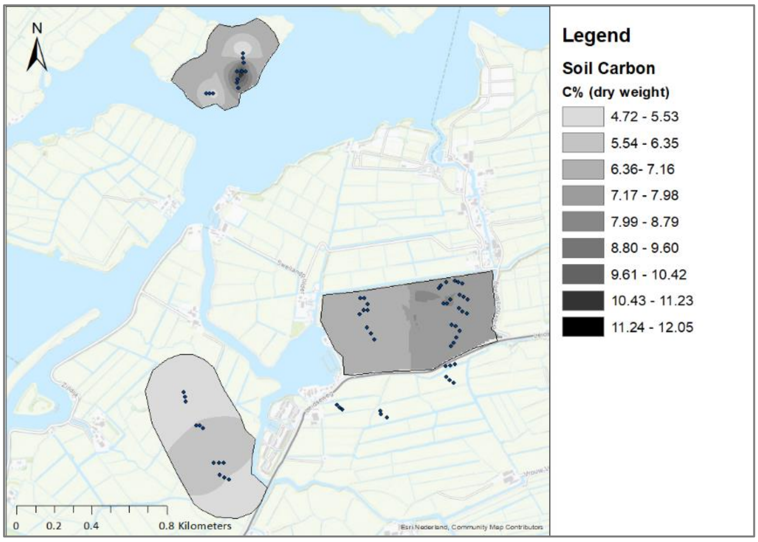


Figure F.1 Soil C:N ratio based on C% and N%.





Appendix G: NDMS figures

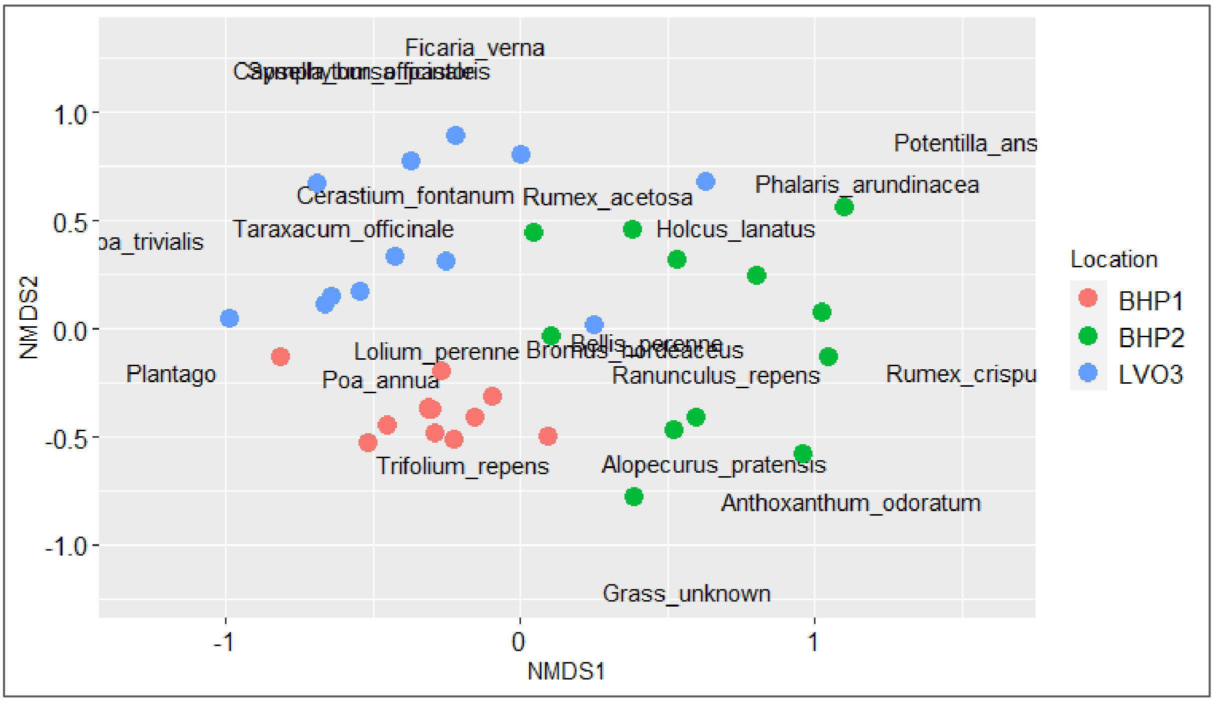


Figure G.1 NMDS of species composition at t0.

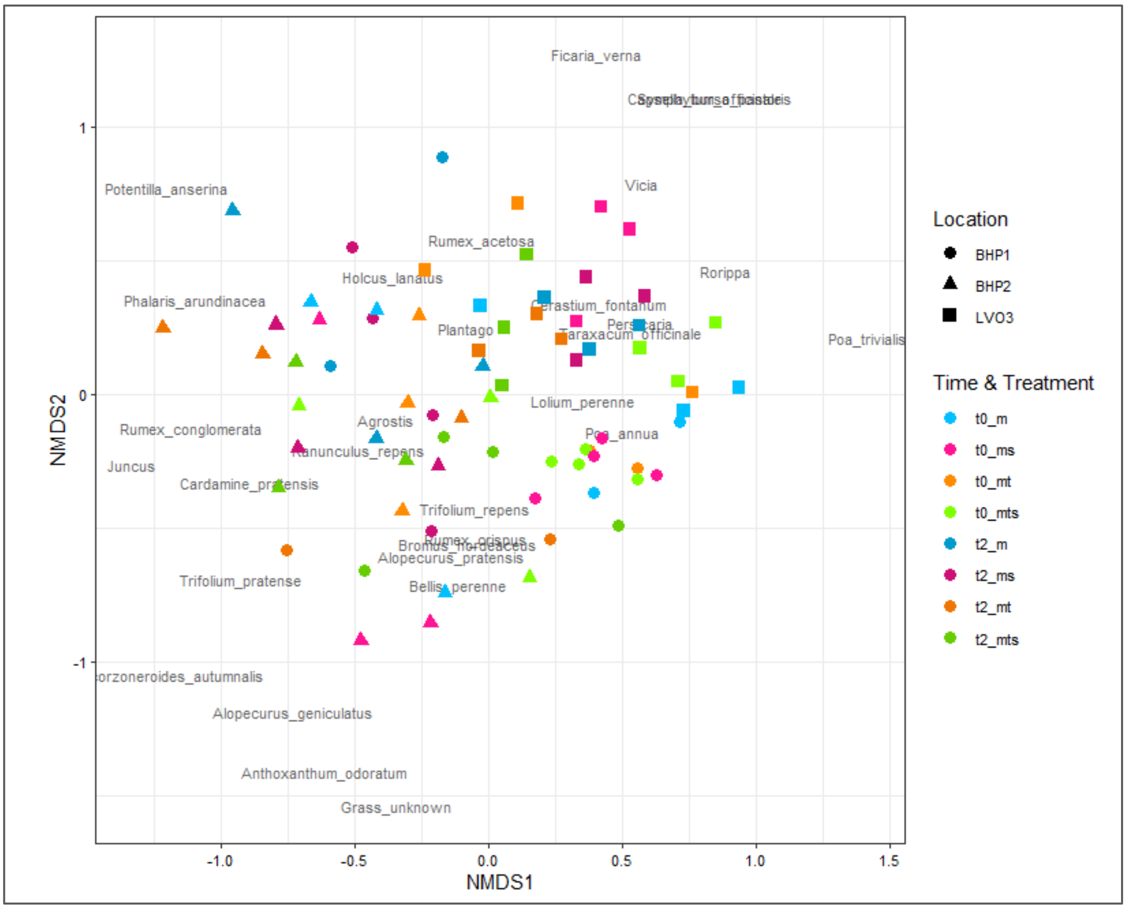


Figure G.2 NMDS of all vegetation data of field experiment. Shapes represent the three experimental sites. Colours indicate moment of recording and treatment applied after t0.

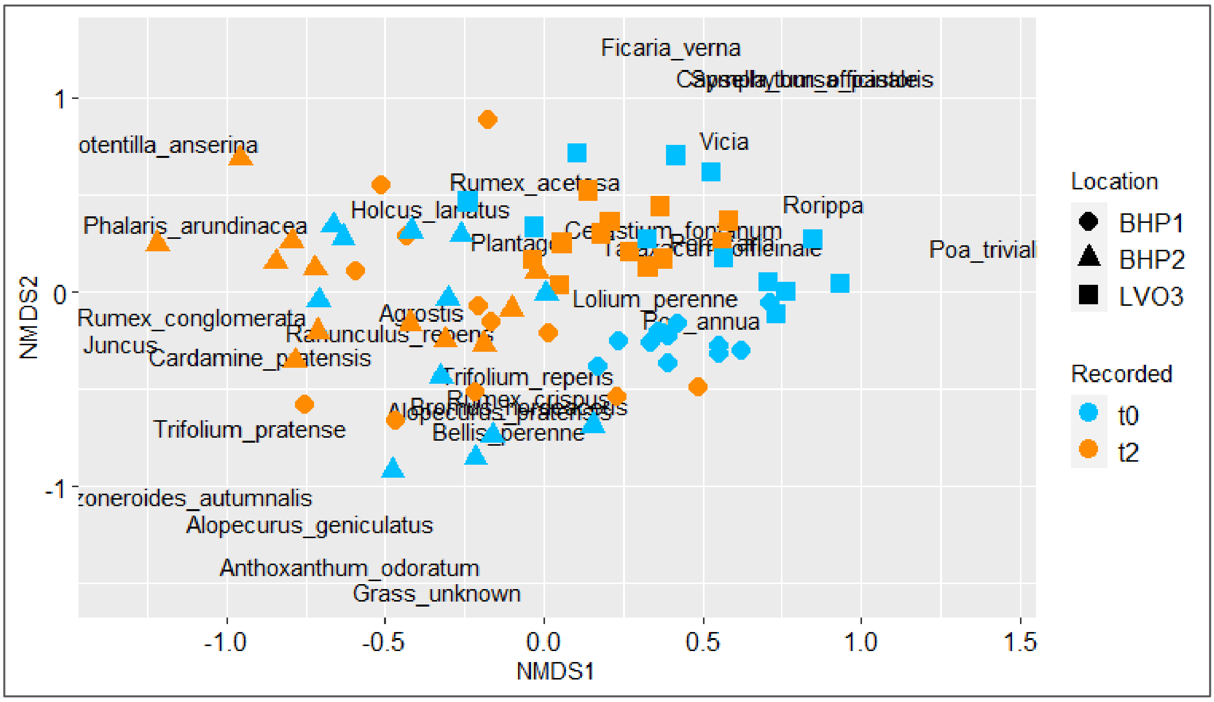


Figure G.3 NMDS of all vegetation data of the field experiment. Shapes represent the three experimental sites. Colours indicate the moment of recording.

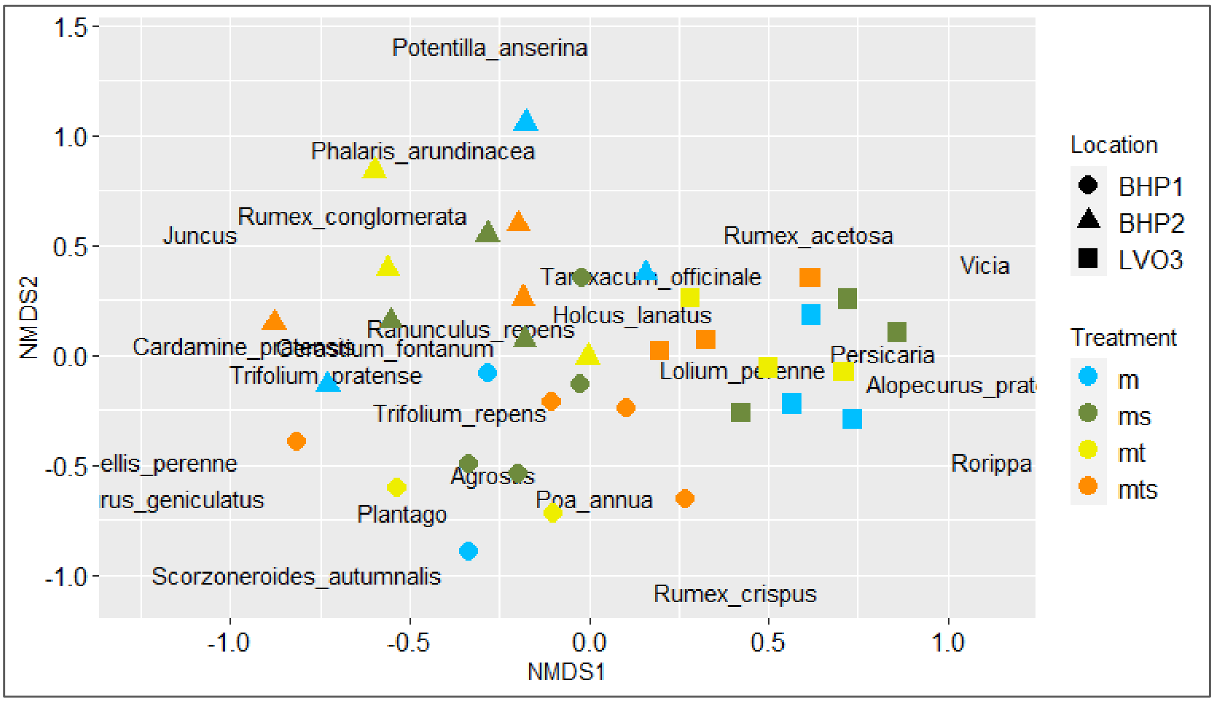


Figure G.4 NMDS of species composition at t2. Shapes represent the three experimental sites. Colours indicate the treatment applied. M = mowing, ms = mowing + sowing, mt = mowing + tillage, mts = mowing + tillage + sowing.

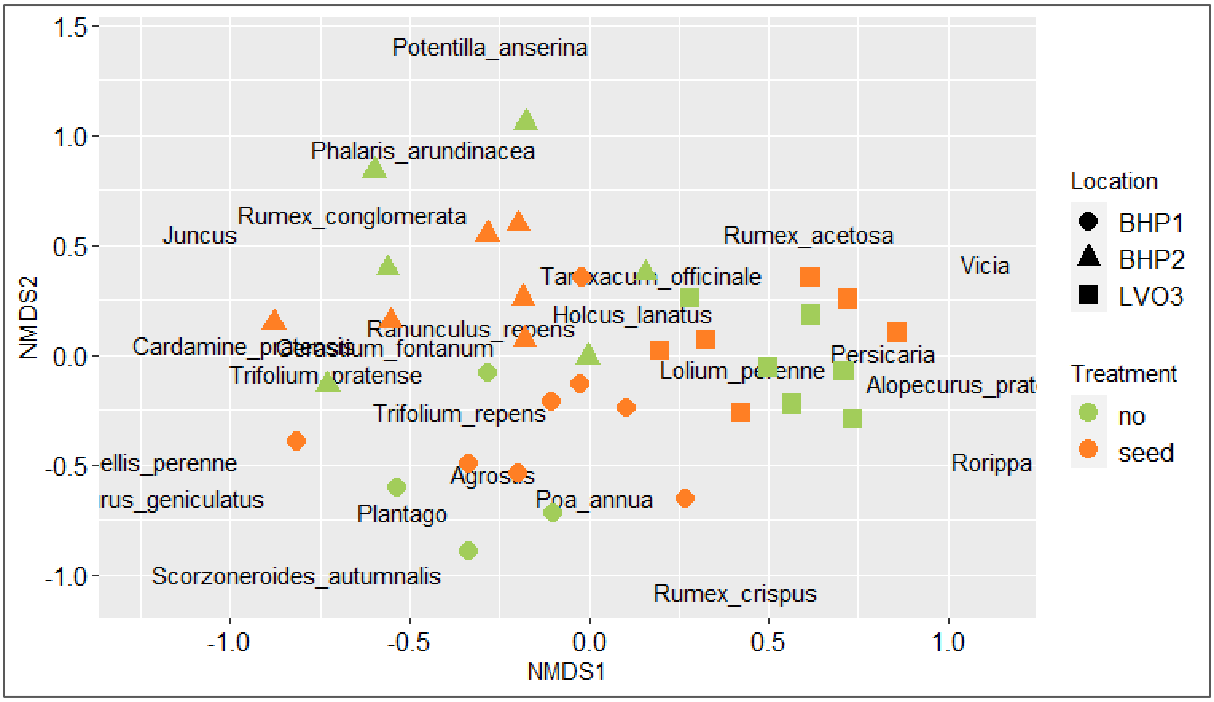


Figure G.5 NMDS of species composition at t2. Shapes represent the three experimental sites. Colours indicate whether plots were sown.

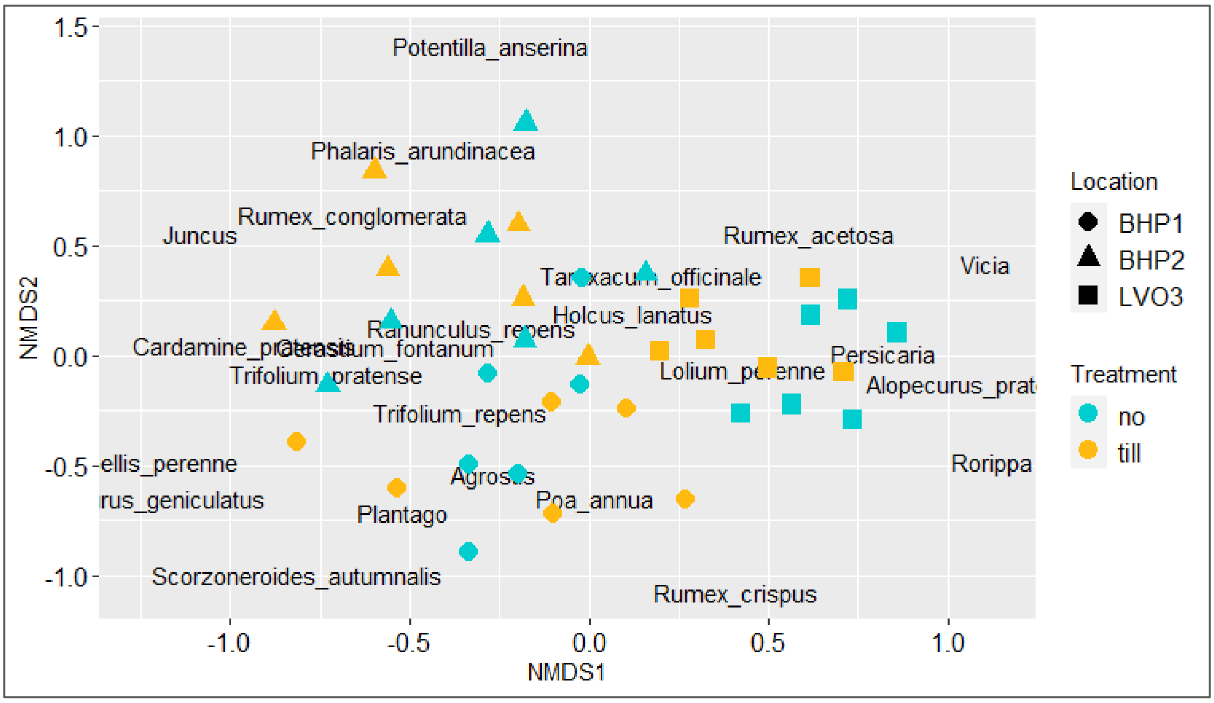


Figure G.6 NMDS of species composition at t2. Shapes represent the three experimental sites. Colours indicate whether tillage was applied.