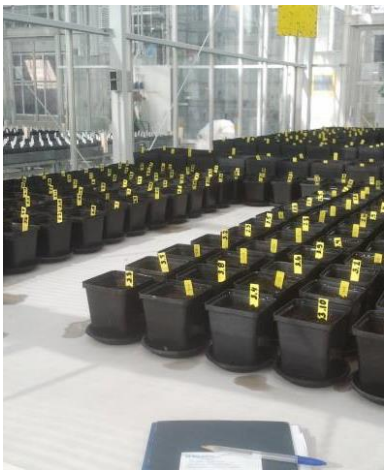
UTRECHT UNIVERSITY

MASTER THESIS SUSTAINABLE DEVELOPMENT (GEO4-2322)

'MIXING SOIL INOCULA AS A RESTORATION MEASURE: ARE THERE SYNERGIES?'



NAME STUDENT:	MRS. P.D. (PAULINE) MELCHERS BSC
STUDENTNUMBER:	3658368
SUPERVISOR UTRECHT UNIVERSITY:	E.R.J. WUBS MSC
SUPERVISOR NIOO-KNAW:	PROF. DR. IR. M.G. RIETKERK
DATE:	1 JULY 2015

ABSTRACT

The composition of soil communities can strongly affect the growth rate and the composition of grassland plant communities, as the soil community changes during succession. However, more research is needed on the most suitable soil (mixture) for soil transplantation as a restoration measure for species-rich grasslands. In this study, the effect of soil communities from different successional stages on the growth of target plants for nature restoration and non-target plants (weeds) was tested by using inoculation. Soil was collected at arable lands, grasslands, and heathlands. Two soil types were mixed (e.g. grassland soil and heathland soil), after which the effect of the mixture on the plant community was determined. Possible synergistic effects between soil communities could then be observed by comparing mixed inocula to pure inocula. This has received little experimental testing. Introducing soil mixtures with a synergistic effect on the plant community could then be a helpful tool for increasing succession from an arable land to grasslands.

It was observed that pots inoculated with 25 percent of grassland soil mixed with 75 percent of heathland soil created a surprising synergistic effect, where the target biomass was higher than expected based on the pure inocula. Furthermore, it was found that pots inoculated with 100 percent heathland soil contained a higher percentage of target biomass at the end of the experiment than pots with other inocula. It can therefore be concluded that soil mixtures containing a high percentage of heathland soils and little grassland soil can enhance the nature restoration of grasslands, where a mixture with 25 percent of grassland soil and 75 percent of heathland soil was most effective due to its synergistic effect on the target biomass.

TABLE OF CONTENTS

1. INTRODUCTION

In Europe, grasslands originate in traditional agricultural landscapes, i.g. in the Netherlands (Veen et al., 2009). However, from 1930 till 1990 agricultural intensification associated with fertilizer application on Dutch lands lead to a constant decline in biodiversity thus turning species-rich grasslands into species-poor communities (Bakker, 1987; Bakker & Berendse, 1999; Dorp, 1996; Smit, 2008). 'Intensive agriculture has resulted in the loss of biodiversity and the specialist flora and fauna associated with the semi-natural grasslands of low-intensity pastoral systems throughout northwest Europe'(Walker et al., 2004). Other factors that have contributed to the decline in biodiversity, are falling water tables, the fragmentation of nature areas, and the acidification of ecosystems. Species-rich grasslands are therefore considered to be rare or even regionally extinct (Dorp, 1996).

The abandonment of agricultural land in Europe, that has been taking place more and more over recent decades, creates opportunities for nature conversion or restoration (Keenleyside et al., 2010; SOER, 2010). After abandonment of these lands, it is usually attempted to convert them into species-rich grasslands in order to restore plant species diversity' (Kardol et al., 2009, p.258). Even though restoring species-rich grassland can be seen as developing an ecosystem without reaching the succesional endstage of a forest ecosystem in a temperate climate, it is a desirable target for many conservation bodies because it is an opportunity to contribute to the preservation of biodiversity. Furthermore, it counteracts the loss of species-rich grasslands, which are being regarded as endangered (Harris, 2008; Török, 2011; Walker et al., 2004; Webb, 1998). Grasslands with higher plant diversity are also perceived as aesthetic more valuable that species-poor grasslands (Lindemann-Matthies et al., 2010). Three problems can be identified that hamper the restoration of species-rich grasslands on former agricultural lands.

Firstly, high soil fertility is considered to be a major constraint for the restoration of former agricultural lands (Kardol et al., 2009; Tsiafouli et al., 2014). Farming systems in the Netherlands are intensive compared to other European countries (Bakker & Berendse, 1999), which has multiple consequences for the state of the land. The use of artificial fertilizers leads to a high nutrient content, which forms a major constraint for the development of species-rich grasslands and heathlands, as it favors fast-growing competitive plant species. This results in the dominance of early-successional and weedy plant species, which makes it difficult for later-successional plant species to colonize the habitat (Kardol, 2007). The dominance of these fast-growing plant species leads to the existence of a low-diversity ecosystem (Huenneke et al., 1990; Kardol, 2007; Kardol et al., 2009). Measures should therefore be taken to overcome the problem of the high fertility when restoring species-rich grassland on former agricultural lands.

Secondly, the establishment of late-successional grassland plant species is also dependent on the presence of their seeds, e.g. through dispersal or recruitment from the seedbank. The soil in former agricultural land mostly doesn't contain the seeds of later-successional grassland plant species (target species), as agricultural practices lead to the elimination and depletion of these species from the seed bank (Bakker & Berendse, 1999; Bekker et al., 1997; Kardol, 2007). Dispersal or transportation of the seeds of late-successional grassland plant species from other sites is therefore needed in order to successfully realize the restoration of species-rich grasslands (Bakker & Berendse, 1999).

Lastly, agricultural practices have affected the structure of the soil and the soil community. The use of heavy machinery in agricultural systems resulted in compaction soil, which negatively influences the soil productivity due to a decrease in soil aeration and reduced root growth due to high penetration resistance. It also leads to the restricted uptake of water and nutrients (Hakansson

& Voorhees, 1997). The cultivation practice of deep ploughing, being referred to as tillage, also alters the availability of nutrients by distributing plant residue and nutrients throughout the plough layer. Both heavy machinery and deep ploughing affect the bacterial community that is able to react to these changes due to its faster dispersal capacity than fungi, while the fungal growth is being inhibited because of the mechanical disturbance of their hyphae (Jansa, 2002; Frey et al., 2009; Jansa et al., 2003; Neher, 1999). Tillage thus leads to a disruption of the soil community by the absence or low-abundance of important components in the soil community, such as fungi, while other organisms increase in abundance (e.g. bacteria) (Jaunatre et al., 2014; Kladivko, 2001; Tsiafouli et al., 2014; Van der Wal et al., 2006). The changed soil community will then influence the aboveground plant community, because the soil community affects the plant community in multiple ways. Microbes are components of the soil community that determine nutrient availability and thus the productivity of plants in natural ecosystems (Van der Heijden et al., 1998). The fungi in the soil, together with bacteria, drive carbon and nutrient availability by decomposition of organic material, therefore stimulating growth of plants (Bardgett & Wardle, 2010; Kardol, 2007; Wardle et al., 2004). Some fungi increase the efficiency of the nutrient exploitation by plants by colonizing plant roots and supplying them with nutrients (Neher, 1999). For example, it was found that arbuscular mycorrhizal fungi (AMF) facilitate plants in mid- and late-succession stages. On the other hand, dwarf shrubs species associate with ericoid mycorrhizas, whereas early succession species are mostly non-mycorrhizal. Due to the different fungi preferences during the successional stages, it can be suggested that fungi contribute to succession (Read, 1991; Read, 1994). Furthermore, larger soil organisms, including nematodes and mites, influence the amount of nutrient mineralization taking place by predating the microbial community and also contribute to decomposition (Bardgett & Wardle, 2010; Neher, 1999). Soil organisms not only determine the decomposition processes and nutrient dynamics in the soil, but can also affect the plant community through root-associated mechanisms. Examples of root-associated organisms are root herbivores, pathogens, and symbiotic mutualists (Bardgett & Wardle, 2010; Wardle et al., 2004).

It can be concluded that soil communities have an impact on the composition, productivity, and diversity of the vegetation. As agricultural practices have disturbed the soil community, restoration or introduction of the soil communities fitting to grassland ecosystems is preferable in order to create suitable conditions for target plant species (De Deyn et al., 2003; Bardgett & Wardle, 2010; Hooper et al., 2000). The important role of soil communities in ecosystems has only recently been emphasized (Bardgett & Wardle, 2010).

To conclude, when restoring former agricultural land to species rich grassland, restoration measures should decrease the fertility of the soil, enhance the establishment of a soil community that matches the successional state of species-rich grasslands, and provide the soil with seedlings of target grassland plant species in order for a species-rich grassland to develop. A very effective way to reduce the amount of nutrients in the soil is top-soil removal, which includes removing the nutrient rich topsoil layer (Geissen et al., 2013). Top-soil removal thus results in a decrease of the fertility of the soil, although it also removes the seeds that are present in the soil (Jaunatre et al., 2014). Furthermore, topsoil removal results in the elimination of the soil community that used to be present in the agricultural system. Top-soil removal could create better circumstances for the establishment of a soil community fitting to species-rich grasslands, due to the nutrient-poor circumstances and the removal of the agricultural soil community. It could therefore positively affect the development towards species-rich grassland (Kardol et al., 2009). However, a soil community suitable for species-rich grassland needs to be transported to the restoration site in order for a target soil community to be present, due to the slow dispersal rate of soil organisms. Combining topsoil removal with transferring soil from other nature areas to the former arable land could help in establishing the target soil community, as the desired organisms are present in the transferred soil (Kardol et al., 2009).

As the aboveground and belowground systems are linked, restoration of the belowground community can be expected to influence the structure and functioning of grassland plant species aboveground, and could thus affect the success of restoration projects (Bardgett & Wardle, 2010). Introduction of the belowground community on land that is being restored could thus positively affect the succession of the aboveground ecosystem (De Deyn et al., 2003). Including the role of soil communities in restoration projects will therefore likely influence the success of such projects (De Deyn et al., 2003). However, current restoration management is focused on decreasing the soil fertility and managing the interactions in aboveground ecosystems, while the important effects of the soil community on the aboveground system are largely being overlooked (Kardol et al., 2006). A better understanding of the role of soil communities in the restoration of nature areas is needed, as this will increase our ability to manage ecosystems responsibly (Harris, 2008; Hooper et al., 2000).

Although the importance of including soil communities in restoration programmes is recognized more and more, understanding of the role of soil communities in the restoration of former agricultural lands is still in its infancy (Bardgett & Wardle, 2010). Kardol et al. (2009, p.258) state that transplanting whole soil communities for the restoration of species-rich grasslands has received little experimental testing. The field experiment of Wubs et al. (2014) showed that soil transplantation of heathland soil can be an effective restoration measure for species-rich vegetation on former arable land, while grassland soil didn't have the desirable effect. As the effects of transplanting different soil communities on the restoration of species-rich vegetation on former arable fields was found to vary, more information about the magnitude of these differences is needed. Furthermore, interactions between different soil communities could create synergistic effects on the growth of target plant species. It would therefore be interesting to study whether or not transplanting soil mixtures could be effective in restoring species-rich grasslands. That is what this study aims to find out, as this greatly affects the extent to which succession can be controlled. Besides generating understanding of the dependency of plants on their soil community, it is also aimed to give recommendations on the restoration management of species-rich grasslands regarding soil transfer measures.

As plants serve as indicators for soil communities (Bardgett & Wardle, 2010) and particularly because they are the main target of restoration programs, it will be interesting to study if the growth rate of different grassland plant species will be affected by the origin of the soil community that is being transferred when restoring grassland ecosystems. It was found that plants are able to influence their abundance by causing changes in the structure of their soil community, which in turn regulates the composition of the plant community (Klironomos, 2002). It could therefore be the case that the succession of the plant community can be determined by the type of soil community it grows upon (e.g. Kardol et al., 2006), however the magnitude of the differences in plant-soil effect between soil types hasn't been studied yet. The research question is therefore formulated as follows:

Are the growth rate and composition of grassland plant communities affected by inoculation with different natural soil communities?

If grassland plant species would be able to grow well on multiple types or mixtures of soils communities, then that will increase the possibilities for soil transfer as a restoration measure for grassland ecosystems. Furthermore, it was found that mixing soil biota influenced community productivity compared to 'pure' soils, which shows the occurrence of synergistic effects (Brandt et al., 2013; Hendriks et al., 2013). These synergies between soil communities would create opportunities for successful nature restoration. This results in a second research question:

Does mixing of soil types in different proportions lead to synergistic effects of the soil on the plant growth of target species for species-rich grasslands compared to the pure inocula containing only one soil type?

Most literature states that the type of soil organisms in a soil, and their interactions, play an important role in determining which plant species grow on that soil (Bardgett & Wardle, 2010). Therefore, it would be most likely that the grassland plant species will develop better on soils from species-rich grassland, as they are more similar and will contain the most suitable soil community.

2. METHODOLOGY

2.1 INOCULATION

In the main experiment, soil from donor sites was inoculated into a common background soil in order to neutralize the difference in environmental conditions among the soil inocula (e.g. the fertility). Consequently, the soil community was considered to be the primary cause for differences in plant growth and plant composition that were observed. A method that is often used is mixing small amounts of live inoculum soil with sterilized background soil (Van de Voorde et al., 2012). The background soil that was used, was gathered at the Reijerscamp, which is an early-successional grassland (out of agricultural production since 2006). The location of the Reijerscamp area can be seen in appendix 1. According to measurements in 2012, the Reijerscamp soil has an organic matter content of 5.92±1.74 percent, a pH of 5.88, a P-concentration of 78.32 mg/kg, a NO_3 concentration of 0.71 mg/kg, and a NH_4 concentration of 7.23 mg/kg. The soil was sterilized to eliminate the soil community in the Reijerscamp soil.

2.2 STUDY AREA

Different soils were collected in the field on January 23rd 2015 'on sandy or sandy loam glacial deposits in the central part of the Netherlands' (Carbajo et al., 2011, p.6), namely the Veluwe. The nature areas are being governed by multiple entities, i.a. Natuurmonumenten. Three types of soils were collected, namely soil of arable land, soil of grasslands and soil of heathlands. Of each type of soil (heathland, grassland, ex-arable land), soil was collected at three different fields, which results in a total of nine fields from which soil will be included in this study (appendix 1). The fields were grouped in three couples based on their location. Each group consisted of one grassland, one heathland and one arable field. In each field, the upper 10 to 15 cm of the soil was taken, where most of the soil community is concentrated (Jaunatre et al., 2014). Soil cores were collected at the four corners of a 5x5 meter square, minimally 20 meter from the edge of the field (Carbajo et al., 2011). At each corner, 5 kg of soil was gathered. All the soil from one field was then mixed in a proportion based on their dry weight, which resulted in a total of around 20 kg of soil per field. Before starting the experiment, the conditions of the soil per field were examined by analyzing soil samples.

2.3 EXPERIMENTAL DESIGN

The proportion of the mixture was 1:9 of inoculum soil to sterilized soil. The inocula contained either one type of soil ('pure' inoculum) or a mixture of different types of inocula (table 1). For example, soil of a grassland and soil of a heathland could be combined in a 3:1 proportion to form a mixed inoculum, after which they were mixed with sterilized soil in a 1:9 proportion. The types of treatments that were applied, are:

- Field type (3x3): soil from three grassland, three heathlands, and three arable fields. Each grassland field was coupled to a heathland field and an arable field, creating a couple. This resulted in three couples and thus a total of nine mixtures (appendix 1).
- Percentages of the two types of soils in inocula, consisting of five options. An example of the percentage options can be seen in table 1, which shows the possible mixtures for a combination of grassland with heathland soil. These percentages were also used for mixtures of arable with grassland soil and mixtures with arable and heathland soil.

Of each treatment there were four replica's, which resulted in a total of 180 pots (9 field pairs x 5 inocula mixing levels x4 replicas).

Table 1: Example of inocula treatments for grassland (G) – heathland (H) mixtures of the first couple number (G1/H1) with their names. These percentages were also used for mixtures of arable with grassland soil and mixtures with arable and heathland soil.

Percentage of grassland soil in inocula	Percentage of heathland soil in inocula	Name of the treatment
100	0	G1-100/H1-0
75	25	G1-75/H1-25
50	50	G1-50/H1-50
25	75	G1-25/H1-75
0	100	G1-0/H1-100

The inoculated soil was divided over pots of 20 cm long and 20 cm wide, with a depth of 15 cm. Seeds were provided by specialized commercial suppliers: Cruydthoeck in the Netherlands, and B&T World seeds in France. The seeds were sterilized with a 5 percent bleach solution, after which seedlings were grown on small moistened glass beads in a climate chamber with 12 hour days at 20 °C and 15 °C at night. Of each plant species (table 2), two individuals were planted in each pot, leading to a total of 12 plants per pot. The positions of each plant species in each pot was randomly assigned, where all positions were situated in a circle. The selected species include both target species (late-successional grassland plant species and dry-heathland plant species) and non-target species (early-successional species and weeds).
The pots with all the plants were then placed in a greenhouse, in which the temperature was 21 °C at day and 16 °C at night, with 16 hours of daylight and an average relative humidity level of 60%. The pots were placed randomly within three blocks on a greenhouse bench, where pots belonging to the same couple number were grouped together. Some distance (20 cm) was left between the pots, in order to create space for the plants to grow, and to eliminate shading effects between pots. The plants were grown for two months on these soils, in which they were watered three times a week with regular tap water.

Table 2: Plant species that are included in the experiment (target and non-target) and their successional stage

Species	Target/Non-target	Group
Lolium perenne	Non-target	Early-successional/weed
Crepis capillaris	Non-target	Early-successional/weed
Myosotis arvensis	Non-target	Early-successional/weed
Campanula rotundifolia	Target	Late-succ. grassland
Arnica montana	Target	Late-succ. grassland
Festuca filiformis	Target	Dry-heathland

2.4 SOIL CONDITIONS

The acidity, nutrient content and the organic matter content were measured for each of the collected soils. Before these parameters were measured, soil samples were sieved over a 1 cm mesh, so that roots and stones were removed. Subsamples of the different types of soils were taken, which were then dried in an oven for five days, with a temperature of 40 °C. For the pH-measurement, ten grams of dry soil was dissolved in 25 ml of demineralized water, after which the solution was shaken mechanically for at least two hours at 250 rpm. After that, the pH in the suspension was determined by using a pH-meter.

The dried soil (five days at 40 °C) was also used to determine the inorganic N, inorganic P, and organic matter content. The Griess-Ilosvay method determined the amount of nitrate in the soil, in which the reagent potassium chloride extracts the nitrogen from the soil: the K^+ particles replace the ammonium in the soil, making them available for measurement, while the high Cl^- concentration effectively extracts nitrate. The obtained ammonium and nitrate were measured colorimetrically at a wavelength of 520 nm (Keeney & Nelson, 1982) by using a SEAL QuAAtro Segmented Flow Analysis (SFA) system. The nitrogen content in the soil material could then be calculated according to:

N (mg/kg) = C / (1000/V) * (1000/W) (eqn 1)

C = the concentration of nitrogen (N-(NO3+NO2) or N-(NH4)) in the soil extract (mg/L)
V = the volume of potassium chloride 1M used for extraction (mL)
W = the exact weight of dry soil used for extraction (g)

Furthermore, the Olsen P test was used to measure the amount of inorganic phosphorus in the soil extract which is available to plants, meaning that $NaHCO_3$ was used to extract P from the soil-water solution. This mechanism is based on an increased solubility of calcium phosphates as a result of lowering the Ca^{2+} activity in the solution and ionic competition of HCO_3^-, CO_3^{2-} and OH^- ions for phosphate adsorbed on the surface of soil particles. The phosphate concentration was determined by spectrophotometry at a wavelength of 880 nm (Olsen et al., 1954) by using a SEAL QuAAtro Segmented Flow Analysis (SFA) system. The amount of available phosphorus in the soil could then be calculated according to:

P (mg/kg) = (C / W) * 250 (eqn 2)

C = the concentration of phosphorus in the soil extract (mg/L)
W = the exact weight of dry soil used for extraction (g)

The organic matter content of the soil was determined by drying the soil in an oven for 24 hours at 105 degrees Celsius, and subsequently by burning the dry soil for 24 hours at 430 degrees Celsius, where the weights of the dry and ashed soils were both determined. The organic matter content (OMC) can then be determined according to:

OMC (g 100 g-1 dry soil) = 100 * ((dry soil - ashed soil) / dry soil) (eqn 3)

Finally, the net effect of the soil community on the target and non-target plants was measured in a separate pot experiment. The species *Crepis capillaris* was selected to represent the non-target community and *Arnica montana* represented the target community. Per pot, three plant species were planted of one plant species, with a total of 10 pots per plant species per field type. Of those 10 pots, half contained a sterile field inoculum, representing a reference situation, while the other half contained a living inoculum. In all cases, soil inoculation took place in a 1:9 proportion, with the same background soil as before. This resulted in a second experiment of 180 pots (2 test species x 9 fields x 2 inoculum treatments (live vs. sterile) x 5 replicates). The pots in this small experiment were 11 cm long and 11 cm wide, with a depth of 12 cm and a content of one kilogram of soil (dry weight).

The pots were placed in the greenhouse, where the pots for each field were grouped together in a randomized order. The environmental conditions are the same as in the main experiment (see section 2.3).

After seven weeks, the pots with non-sterilized field inoculum were compared to pots inoculated with sterilized soil according to:

NE (non-target) = B1 (C.cap) – B2 (C.cap) (eqn 4)
NE (target) = B1 (A.mon) – B2 (A.mon) (eqn 5)

NE = net effect of the soil on the plant species
B1 = biomass on non-sterilized soil
B2 = biomass for the reference situation

For each comparison the same inoculum sample in both sterilized and non-sterilized treatments were compared.

2.5 SHOOT BIOMASS

After two months, the shoot biomass of the plants was determined. Shoots were clipped at ground level, after which they were dried in an oven for two days at 75 degrees Celsius (Kardol et al., 2006). Finally, the biomasses of the dry shoots were weighed.

Per pot, the total amount of target and non-target biomass was determined, with the target community consisting of *Campanula rotundifolia*, *Arnica montana*, *Festuca filiformis*, and the non-target community being *Lolium perenne*, *Crepis capillaris*, and *Myosotis arvensis*. Furthermore it was calculated what percentage of the biomass per pot consisted of target versus non-target biomass.

2.6 STATISTICAL METHODOLOGY

All the data, both of the environmental conditions of the soil, and of the shoot biomass, were gathered in IBM SPSS Statistics 22 (2013) and then analyzed. The unstandardized residuals of the plant biomasses were tested for normality by using a Shapiro-Wilk test and inspected visually using histograms with a normal distribution curve. As not all of the unstandardized residuals from the biomasses per plant species were found to obey normality (minor deviations from the normal distribution curve were found for *Crepis capillaris* and *Campanula rotundifolia*), a Kruskal Wallis test was performed to check the outcomes of the parametric analyses. The Kruskal Wallis test showed the same results as the outcomes found in section 3 – thus for simplicity the original parametric test are presented in the results. The parametric tests include ANOVA's and MANOVA's for study the growth rate between the inocula types. A Pearson correlation test was executed in order to study correlations between the growth rate of different species within the plant community. The dependent variables consisted of the absolute or relative plant abundances (in gram biomass or percentages), while the independent variables consisted of the type of inoculum and couple number.

In order to determine if a synergistic effect can be found in one of the mixtures of inocula, expected results for the mixed inocula were calculated from the pure inocula which could then be compared to the observed biomass (section 3.3). The expected results were based on the observed biomass for the pure inocula (A100, G100, and H100) and the percentage of each soil type in the mixture (25, 50 or 75 percent), after which the net difference between the expected and observed biomass was determined. For example, the expected target biomass for the A25-G75 mixture was calculated according to:

Expected target biomass (A25-G75) = (0.25 * observed target biomass (A100)) +
(0.75 * observed target biomass (G100)) (eqn 6)

3. RESULTS

3.1 TARGET VERSUS NON-TARGET

When looking at the total biomass per pot (both target and non-target species combined) in figure 1, no clear differences seem to be present between the types of inoculum. A One-Way ANOVA with a Tukey post-hoc test showed no overall significant effect of the type of inoculum on the total biomass ($F_{11, 166}$=1.269; p=0.246).

The target biomass had a lower percentage of biomass per pot than the non-target biomass, with an overall mean (±SD) of 7.22±2.62% for the target biomass percentage. The target plant species were therefore subordinate in the plant community. Although the target biomass had a much lower share in the total biomass per pot than the non-target biomass (92.78±2.62%), the different types of inoculum did lead to changes in the composition of the plant communities. The One-Way ANOVA shows that there is an overall effect of the type of inoculum on percentage biomass of target species ($F_{11,166}$ = 4.564; p<0.0005). The Tukey post-hoc test shows that G25-H75 and H100 differ significantly from the other inocula (p<0.05), except for G75-H25, G50-H50, H75-A25, and H50-A50 (figure 2).

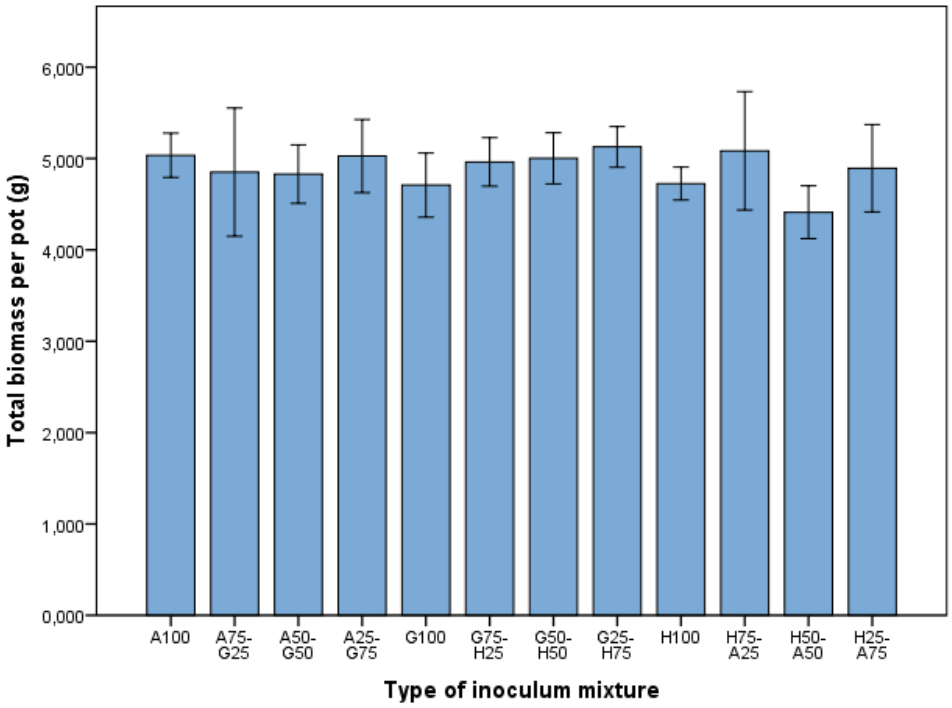


Figure 1: *Total plant biomass per pot (g) for the different types of inoculums. Error bars show 95% confidence intervals. For an explanation of the inocula codes, see table 1 in section 2.3*

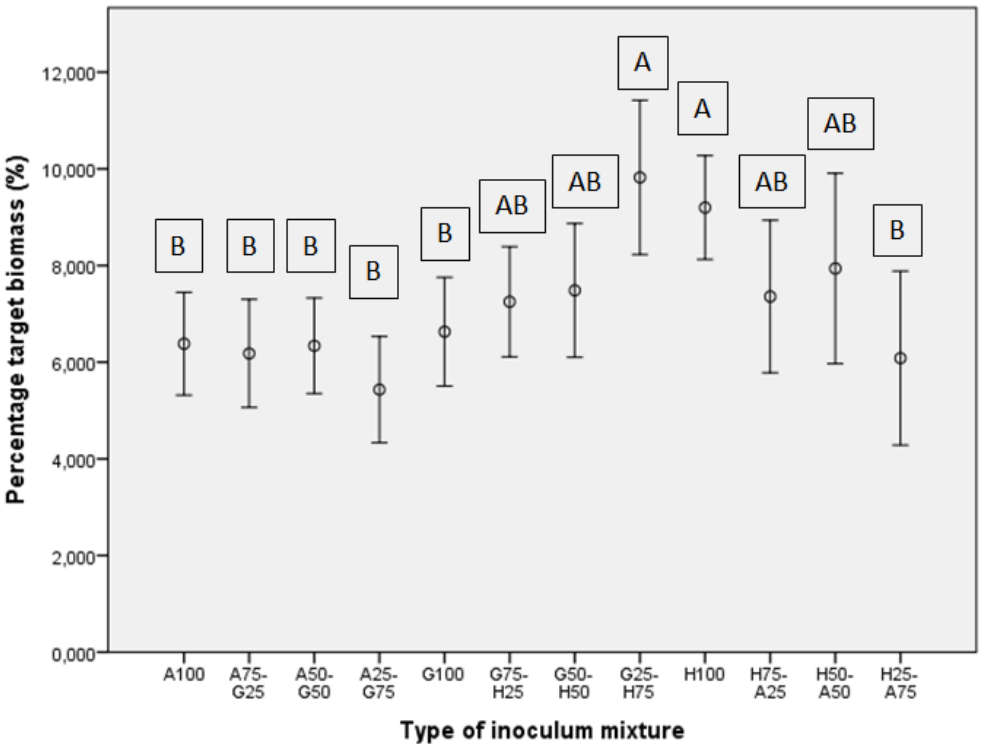


Figure 2: *Percentage target biomass per pot (%) for the different types of inocula. Grouping based on a Tukey post-hoc test, where different letters indicate significant differences. Error bars show 95% significance levels for trends. For an explanation of the inocula codes, see table 1 in section 2.3.*

3.2 COMPOSITION OF THE PLANT COMMUNITY

A MANOVA was executed in order to determine if the type of inoculum significantly affects the biomass of the different plant species, which was found to be the case (Pillai's trace=0.761; $p<0.0005$). For the plant species individually, it could be seen that only *Lolium perenne* wasn't significantly influenced by the type of inoculum ($F=0.510$; $p=0.985$), whereas the other plant species were affected significantly ($F=1.892$-8.783; $p=0.0005$-0.044).

Figure 3 shows the biomass percentage of each plant species per pot. It can be observed that a correlation is present between *Crepis capillaris* and *Myosotis arvensis* ($p<0.0005$; $F= -0.647$), between *Lolium perenne* and *Myosotis arvensis* ($r = -0.292$, $p<0.0005$;), and between *Crepis capillaris* and *Lolium perenne* ($r= -0.516$, $p<0.0005$; table 3). The relative abundance of non-target plant species are therefore negatively correlated. The outcomes for the correlations between other plant species can be found in table 3. It can be seen that *Myosotis arvensis* is negatively correlated to all of the other plant species, both target and non-target. Furthermore, *Arnica montana* is positively correlated with the abundance of *Campanula rotundifolia* ($r=0.217$; $p=0.004$) and *Festuca filiformis* ($r=0.236$; $p=0.001$), which are all considered to be target species.

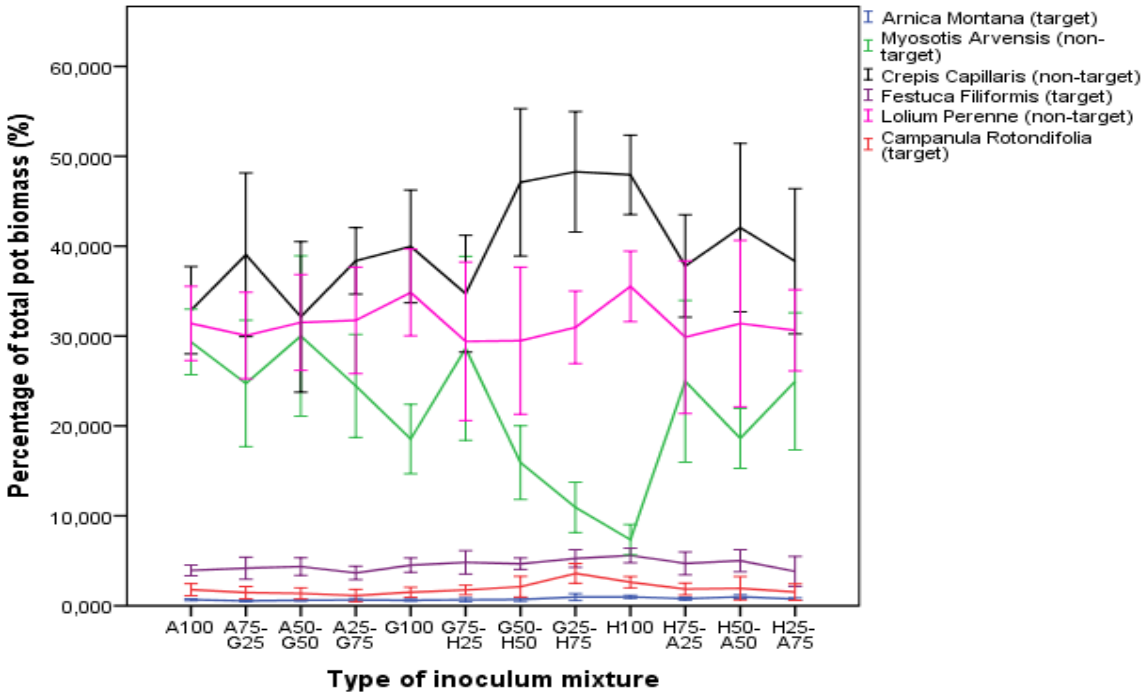


Figure 3: *Biomass percentage of each plant species per pot for the different types of inocula. Error bars show 95% significance levels for trends. For an explanation of the inocula codes, see table 1 in section 2.3. Lines are displayed in the graph as visual aid.*

Table 3: *Correlations between the biomass percentages of species in the plant community (N=178). A p-value lower than 0.05, or 0.01 in some cases (**), represent a significant correlation. The Pearson correlation shows the magnitude of the correlation.*

		Myosotis arvensis	***Crepis capillaris***	***Lolium perenne***	***Festuca filiformis***	***Campanula rotondifolia***	***Arnica montana***
Myosotis arvensis	Pearson Correlation		-.647**	-.292**	-.233**	-.225**	-.191*
	Sig. (2-tailed)		<0.0005	<0.0005	0.002	0.003	0.01
Crepis capillaris	Pearson Correlation	-.647**		-.516**	.204**	-0.006	0.083
	Sig. (2-tailed)	<0.0005		<0.0005	0.006	0.941	0.269
Lolium perenne	Pearson Correlation	-.292**	-.516**		-.181*	0.097	0.015
	Sig. (2-tailed)	<0.00050	<0.0005		0.015	0.198	0.839
Festuca filiformis	Pearson Correlation	-.233**	.204**	-.181*		0.124	.236**
	Sig. (2-tailed)	0.002	0.006	0.015		0.100	0.001
Campanula rotondifolia	Pearson Correlation	-.225**	-0.006	0.097	0.124		.217**
	Sig. (2-tailed)	0.003	0.941	0.198	0.1		0.004
Arnica montana	Pearson Correlation	-.191*	0.083	0.015	.236**	.217**	
	Sig. (2-tailed)	0.01	0.269	0.839	0.001	0.004	

3.3 EXPECTED VERSUS OBSERVED MIXTURE BIOMASSES

In order to determine if a synergistic effect can be found in one of the mixtures of inocula, the calculated expected biomasses (see section 2.6) were compared to the observed biomasses, resulting in a net difference. A One-Way ANOVA showed that overall significant differences can be found between the net biomass difference of the types of inoculum for the target biomass ($F_{11, 166}$=2.627; p=0.004). A Tukey post-hoc test shows that G25-H75 differs significantly from all of the other inocula (p<0.05), except for G75-H25 (p=0.456), G50-H50 (p=0.279), and H75-A25 (p=0.151). Furthermore, only G25-H75 was found to be significantly different from zero (figure 4), and thus significantly different from the expected biomass.

The non-target species do not show a clear trend for the differences between the expected and observed biomasses in the pots. On most of the inocula, the observed biomass for non-target species was higher than the expected biomass, but never significantly so. A One-Way ANOVA for the non-target species showed that no overall significant differences can be found between the net biomass difference of the types of inoculum ($F_{11, 166}$=1.617; p=0.098). The Tukey post-hoc test also shows that no significant differences can be found between the types of inocula (p>0.05).

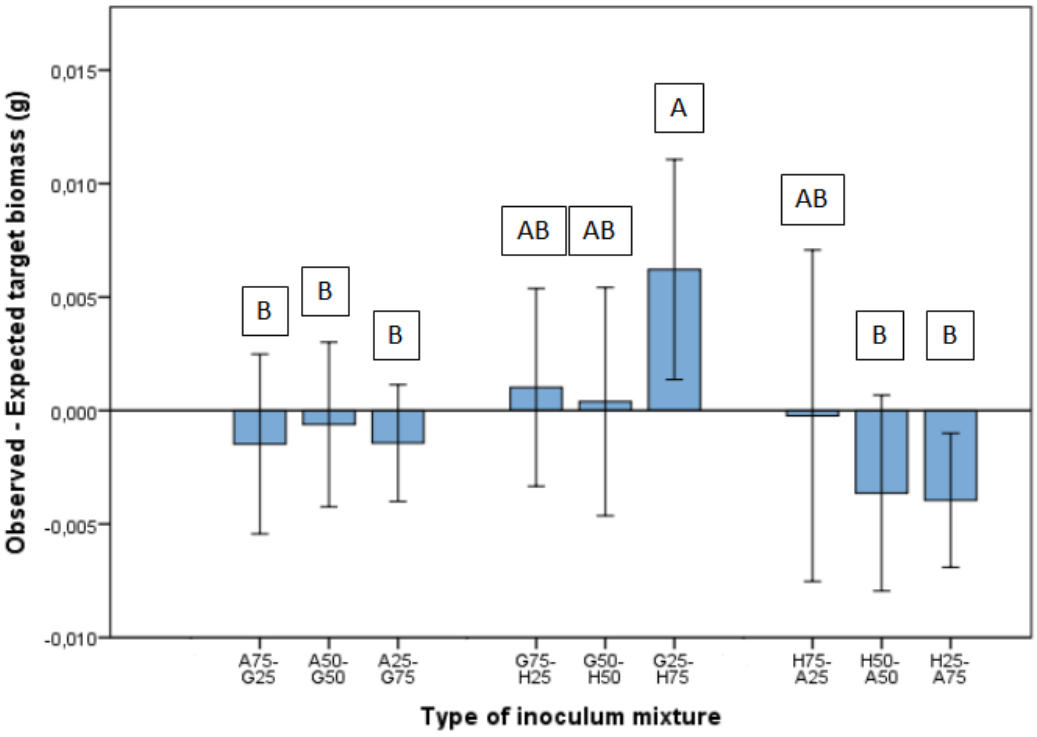


Figure 4: *Net difference between the observed target biomass and the expected target biomass (g) for the different types of inocula. Grouping (A and B) based on a Tukey post-hoc test. Error bars show 95% confidence intervals. For an explanation of the inocula codes, see table 1 in section 2.3, and for a description of the expected biomass calculations see section 2.6.*

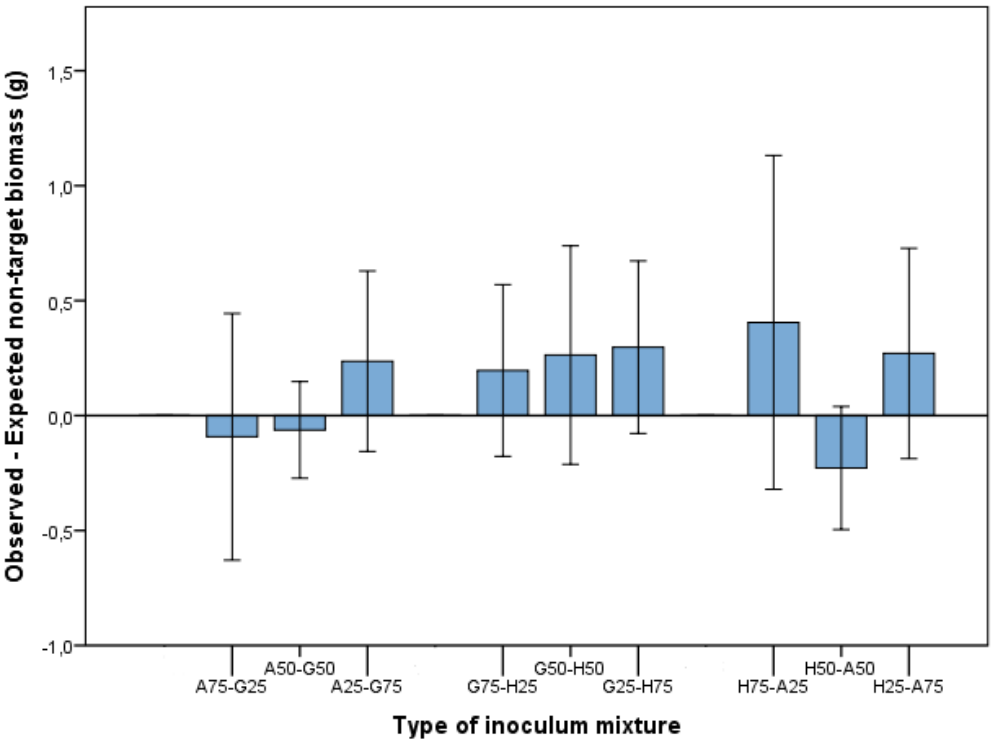


Figure 5: *Net difference between the observed non-target biomass and the expected non-target biomass (g) for the different types of inocula. Error bars show 95% significance levels for trends. For an explanation of the inocula codes, see table 1 in section 2.3, and for a description of the expected biomass calculations see section 2.6*

3.4 COUPLE NUMBER DIFFERENCES

The effect of the couple number on the target biomass illustrates whether the origin of the soil has an effect on the results that were found. As each couple number contains soil from different field sites, possible outliers for a specific field could have influenced the results. The effect of the couple number on the biomass for the target plant species can be seen in figure 6. It can be seen that the second couple number deviates from the other couples, especially at G25-H75 and H75-A25. A univariate analysis shows that the type of inoculum together with the variable for couple number (inoculum type * couple number) has a significant effect on the net difference between the expected and observed target biomass ($F_{22, 142}$=2.112; p=0.005).

For the non-target biomass, the second couple also most strongly deviated from the first and third couple at H75-A25, but the first couple number deviated at the mixtures containing soil from arable land (figure 7). The univariate analysis for the non-target biomass shows that the type of inoculum and the couple number together (inoculum type * couple number) have a significant effect on the non-target biomass ($F_{22, 142}$=7.016; p<0.0005).

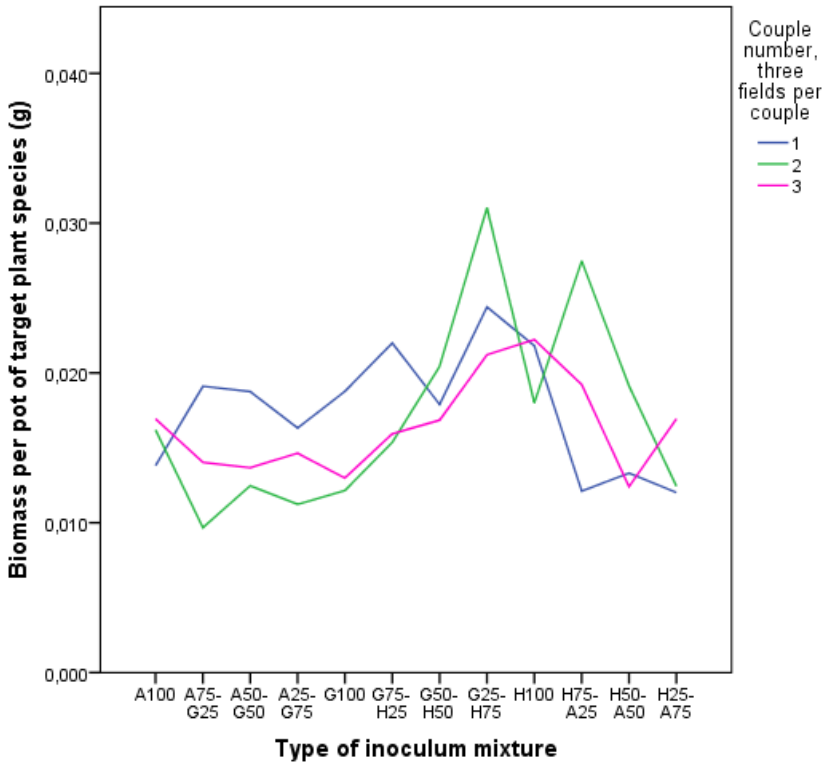


Figure 6: *Target biomass (g) per couple number for the different types of inocula. For an explanation of the inocula codes, see table 1 in section 2.3. Appendix 1 shows the fields that belong to each couple number. Lines are displayed in the graph as visual aid.*

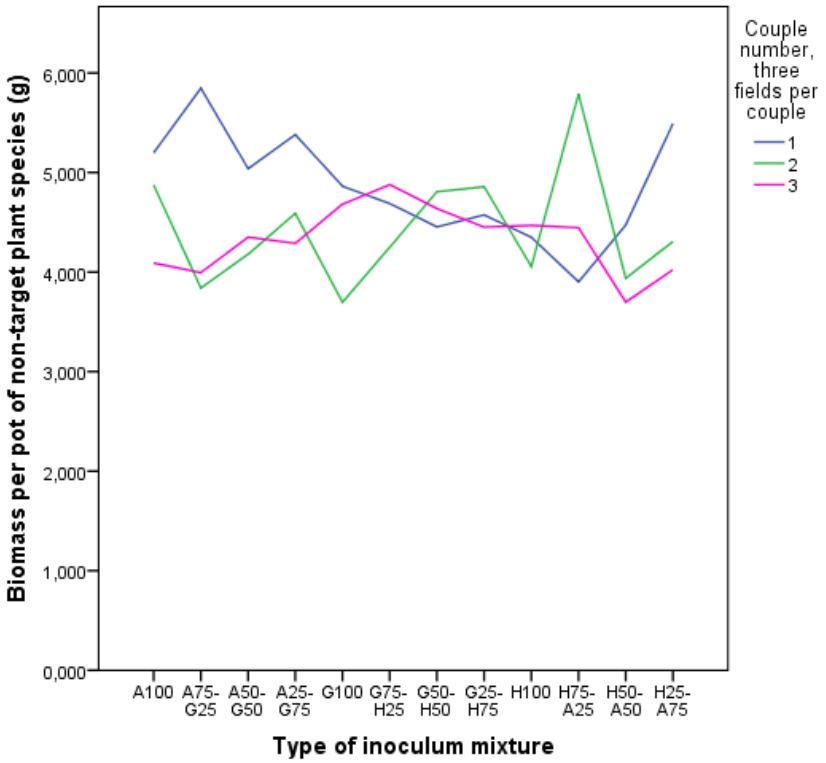


Figure 7: *Non-target biomass (g) per couple number for the different types of inocula. For an explanation of the inocula codes, see table 1 in section 2.3. Appendix 1 shows the fields that belong to each couple number. Lines are displayed in the graph as visual aid.*

3.5 LINKING RESULTS TO SOIL PROPERTIES

The soil properties of the soil collected from the different fields, can be seen in table 4. Arable lands were found to have the highest pH and the highest phosphate concentration in the soil, with a similar organic matter content, NH_4 concentration and NO_3 concentration to late grasslands. The net effect of arable soils on the aboveground biomass of both non-target species (*Crepis capillaris*) and target species (*Arnica montana*) was relatively the most negative compared to the other soil types, only the effect from Sindehoeve on the non-target biomass differed. Late grasslands seem to vary in their soil properties, with values mostly in between the values for the ex-arable and heathland soils. The effect of late grasslands on the aboveground non-target species (*Crepis capillaris*) was found to vary, as both small positive and negative values were found, while their effect on the aboveground target species (*Arnica montana*) is quite negative. Heathland soils have the highest organic matter content and NH_4 concentration, but lowest pH, phosphate concentration, and NO_3 concentration. Heathlands had a relatively positive effect on the aboveground non-target species (*Crepis capillaris*) and a slightly negative effect on the target species (*Arnica montana*) (table 4).

The results in the previous sections show that the inocula of G25-H75 and H100 are significantly different compared to the other types of inoculum. Comparing this to the properties of the soil collected in the field, the soil properties of the heathland soils might have influenced the performance of the plants on H100, as a higher target biomass and a lower non-target biomass was observed (section 3.1). However, for the G25-H75 mixture no clear link can be made between the higher percentage of target biomass per pot and the soil properties. Due to the 1:9 inoculation of the soil, the effects of the soil properties on the plant growth should have been filtered out.

When comparing the net effect of the soil types on the aboveground target and non-target species (table 4) with the observed biomass for *Arnica montana* in the community experiment (figure 8 and 9), no clear link can be made. The same holds for *Crepis capillaris*. The variation in the growth rate of *Arnica montana* and *Crepis capillaris* do not show a clear trend between the net effects of the different types of soil.

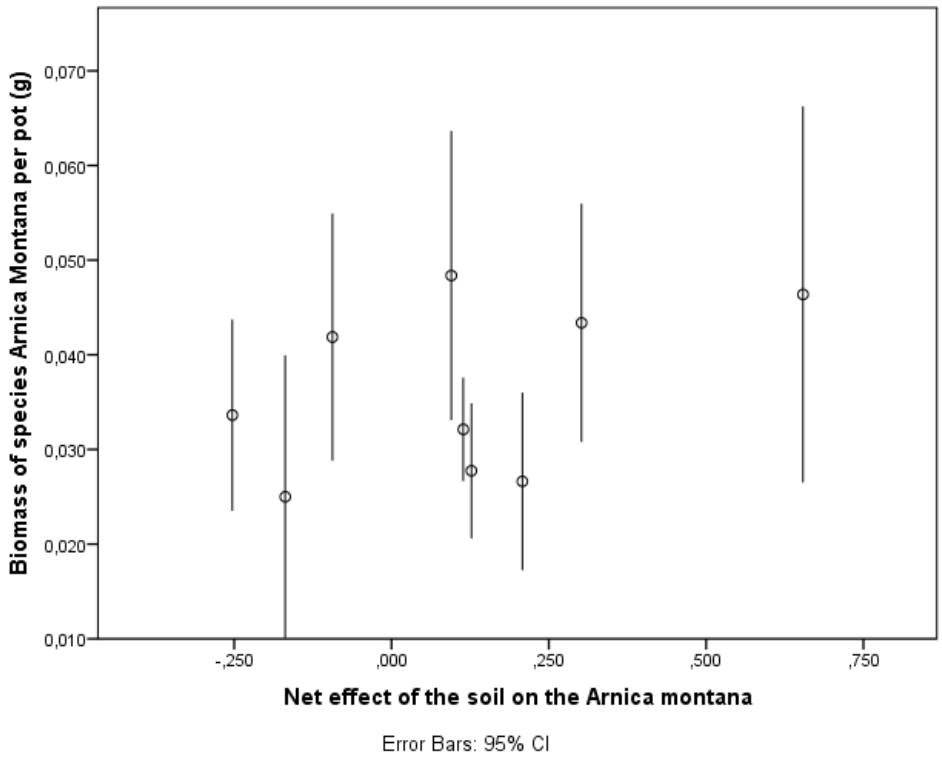


Figure 8: Arnica montana biomass per inoculum type in the community experiment (g) compared to the observed net effect of the soil on Arnica montana (table 4). For an explanation of the inocula codes, see table 1 in section 2.3.

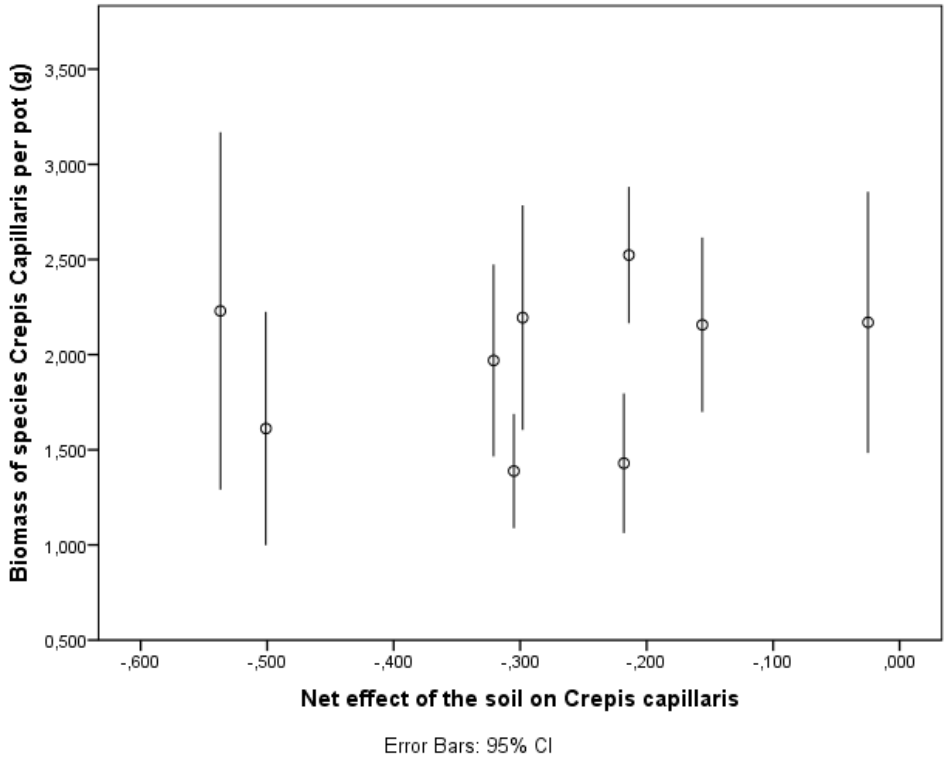


Figure 9: *Crepis capillaris biomass per inoculum type in the community experiment (g) compared to the observed net effect of the soil on Crepis capillaris (table 4). For an explanation of the inocula codes, see table 1 in section 2.3.*

Table 4: Soil properties of the soil collected in the fields, with their standard deviation and the significant differences for the soil properties (Duncan post-hoc test) and for the net effect of the soil on the target and non-target species (Tukey post-hoc test) represented by the letters. Values that show no significant differences are grouped together with the same letter.

Field site	**Type of soil**	**Couple Number**	**Organic matter content (%)**	**Acidity (pH)**	**P concentration (mg/kg)**	**NO_3 (mg/kg)**	**NH_4 (mg/kg)**	**Net effect of soil on aboveground non-target species (C.cap)**	**Net effect of soil on aboveground target species (A.mon)**
Reijerscamp	Arable	1	5.23±0.11 (BC)	5.437±0.070 (G)	53.41±2.54 (CD)	4.24±0.95 (BC)	2.18±0.21 (AB)	-0.094±0.156 (ABC)	-0.321±0.234 (ABC)
Reemsterakker	Arable	2	3.20±0.10 (A)	5.359±0.141 (G)	58.44±2.25 (A)	6.27±2.00 (A)	2.40±0.99 (CD)	-0.253±0.177 (A)	-0.305±0.152 (ABC)
Sindehoeve	Arable	3	5.69±0.30 (C)	4.051±0.006 (C)	120.27±13.43 (E)	6.80±0.29 (D)	2.37±0.08 (AB)	0.208±0.134 (CD)	-0.501±0.159 (AB)
Mosschelse Veld (grassland)	Grassland	1	3.37±0.02 (A)	4.589±0.009 (E)	52.42±1.08 (CD)	5.72±0.52 (CD)	5.14±2.00 (D)	0.114±0.351 (ABC)	-0.537±0.115 (A)
Dennenkamp	Grassland	2	4.66±0.08 (B)	4.722±0.035 (F)	47.19±2.28 (BC)	3.88±0.53 (B)	2.87±0.41 (AB)	0.127±0.370 (BCD)	-0.218±0.114 (CD)
Wolfhezer Veld	Grassland	3	5.07±0.26 (BC)	4.279±0.016 (D)	39.14±3.10 (B)	7.30±1.38 (D)	1.96±0.34 (A)	-0.169±0.091 (B)	-0.298±0.067 (BC)
Mosschelse Veld (heathland)	Heathland	1	6.84±1.44 (D)	3.598±0.020 (B)	2.73±1.20 (A)	0.45±0.15 (A)	3.63±0.19 (BC)	0.302±0.308 (D)	-0.025±0.068 (D)
Reemsterheide	Heathland	2	8.10±0.17 (E)	3.105±0.015 (A)	2.16±0.48 (D)	0.51±0.04 (D)	4.90±0.10 (AB)	0.095±0.180 (ABCD)	-0.156±0.240 (CD)
Doorwerthse Heide	Heathland	3	7.73±0.29 (E)	3.655±0.004 (B)	1.02±0.14 (A)	1.23±0.09 (A)	6.11±0.49 (D)	0.654±0.250 (E)	-0.214±0.130 (CD)

4. DISCUSSION

The results showed that the growth rate and composition of grassland plant communities is significantly different for the types of inoculum with either 100 percent heathland soil or a mixture with 25 percent of grassland soil and 75 percent of heathland soil. For these inocula, the proportion of target species to non-target species was found to be different from the other inocula, with a higher percentage of target biomass. The biomass on the H100 soil was expected to be higher as the soil was found to be more suitable for target species in the field experiment of Wubs et al. (2014). However, the target biomass for the G25-H75 mixture was surprisingly higher than expected, without a significant increase of the non-target biomass. It could therefore be concluded that this type of mixture of inocula has a synergistic effect on the growth of the target species, which are positively affected. Based on this experiment both inocula H100 and G25-H75 seem to be most suitable for increasing the restoration success of grasslands, where G25-H75 would most likely be more suitable as it contained the highest biomass for the target plant species.

It was predicted (section 1) that inocula with grasslands soil would be best for a target community to grow on, due to the presence of AMF. Arbuscular mycorrhizal fungi facilitate plants in mid- and late-succession stages while the non-target species (weeds) are mostly non-mycorrhizal (Read, 1994). However, a higher performance of target species on grassland soil wasn't observed in this experiment. The inoculation of soil from an ecosystem in a later successional stage (heathlands) is found to be a more successful management strategy for the restoration of grasslands. This is contradictory to Kardol et al. (2009), who observed that the introduction of a target soil community didn't enhance nature restoration of grasslands. However, the unfavorable soil conditions at the restoration site may be the cause for the results observed by Kardol et al. (2009), as a mismatch occurred between the abiotic conditions of the transplanted soil and the receptor site.

As the successional state of the soil differs per nature area, this could have played a small role in the effect of the soil inocula on the target and non-target biomass (Bardgett & Wardle, 2010; Wardle et al., 2004). It could be the case that the grassland soil communities were insufficiently to produce beneficial conditions for the target plant species. The importance of the successional state of the field is confirmed by Read (1991, p.387-388), who states that the 'compatibility with approprioate mycorrhizal associates appears to be a key factor determining not just the fitness of the plants but the structure of the whole plant community'. The higher performance of target plant species on inocula with high levels of heathland soil (H100 and G25-H75) might therefore be explainable by the presence of a different type of fungi, namely the ericoid mycorrhizas instead of arbuscular mycorrhizal fungi (Read, 1991), however this cannot be proven based on this experiment. More research is needed before any conclusions can be drawn about the optimal soil community that should be introduced in order to restore grasslands.

When comparing the couple numbers, and thus the origin of the soil, it was found that this significantly affects the biomass for both the target and the non-target community. For the second couple number, the observed biomass deviated from the other two couples, especially the mixtures containing a high concentration of heathland soil. As the Reemsterheide, which is representing heathland soil in the second couple, doesn't show soil properties that are very different compared to the other heathland soils, the soil organisms could be the cause for this effect. It could be the case that the positive effects of H100 and G25-H75 on the target plant species were mainly found due to the outcomes of Reemsterheide, as the results for the other fields seem to be more neutral. The implication for research is therefore that no specific cause can be pointed out for the success of the H100 and G25-H75 inocula. This complicates the

applicability of mixing soil inocula for nature restoration, as it is unclear which field soils will be successful.

Looking at the effects at species level of the experiment, *Crepis capillaris* was the most dominant plant species in the community. The other non-target species were found to negatively correlate with *Crepis capillaris*, especially *Myosotis arvensis*, suggesting competition. More interestingly, *Crepis Capillaris* showed a positive correlation with the target plant species *Arnica montana* and *Festuca filiformis*. This could mean that *Crepis capillaris* suppresses the growth of other non-target species, resulting in more space for the target plant species to grow. It should be noted that in a natural ecosystem the presence of more species could lead to different outcomes regarding the community composition, as the plant community and its interlinkages with the soil community will become more complex with an increasing amount of species.

It is recommended that more research is done to look at the possibilities of using mixtures with low percentages of grassland and high percentages of heathland soils for the restoration of grasslands, as this shows promising results in this experiment. In future studies the organisms that are present in the soil community should be determined, so that the outcomes for plants can be linked to specific soil organisms that are present and thus conclusions can be made about the nature of the synergistic effect that was found.

Overall, it can be concluded that inoculation with mixtures containing 100 percent of heathland soil, or a mixture of 25 percent of grassland soil and 75 percent of heathland soil, have a significant effect on the growth rate and composition of grassland plant communities. This suggests that the resulting plant community can be altered depending on the type of inoculum used during restoration Furthermore, it can be concluded that a mixture of 25 percent of grassland soil and 75 percent of heathland soil show a synergistic effect of the soil on the plant growth of the target plant species compared to the pure inocula.

5. CONCLUSION

It can be concluded that inocula with 100 percent heathland soil, and mixtures with 25 percent grassland and 75 percent heathland soil, contain a higher percentage of target biomass than the other inocula. For the mixture with 25 percent grassland and 75 percent heathland soil, the target biomass was higher than expected, suggesting a synergistic effect of the soil organisms on the target biomass. Furthermore, the data suggest that that the dominant species in the artificial communities, *Crepis capillaris*, affected the biomass of the other plant species in the pots, where *Crepis capillaris* suppressed the growth of other non-target species, potentially resulting in more space for the target plant species to grow.

6. ACKNOWLEDGEMENTS

I would like to thank Jasper Wubs and Max Rietkerk for their supervision and contributions to my thesis. Furthermore, I'm grateful for the practical help I got from Rienke Ruijs, Tom van Heusden, Rebecca Pas, and Eke Hengeveld. Lastly, I thank the Netherlands Institute of Ecology (NIOO-KNAW) for their hospitality and generosity during my internship.

7. LITERATURE

Bakker, J. P. (1987). Restoration of species-rich grassland after a period of fertilizer application. In Andel et al. (Eds.), *Disturbance in grasslands* (pp. 185-200). Springer Netherlands.

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

Bardgett, R. D., & Wardle, D. A. (2010). *Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change* (10-11pp). Oxford: Oxford University Press.

Bekker, R. M., Verweij, G. L., Smith, R. E. N., Reine, R., Bakker, J. P., & Schneider, S. (1997). Soil seed banks in European grasslands: does land use affect regeneration perspectives? *Journal of Applied Ecology*, 1293-1310.

Brandt, A. J., Kroon, H., Reynolds, H. L. & Burns, J. H. (2013). Soil heterogeneity generated by plant–soil feedbacks has implications for species recruitment and coexistence. *Journal of Ecology*, *101*(2), 277-286.

Carbajo, V., Den Braber, B., Van Der Putten, W. H., & De Deyn, G. B. (2011). Enhancement of late successional plants on ex-arable land by soil inoculations. *PLoS ONE*, *6*(7), e21943.

De Deyn, G. B., Raaijmakers, C. E., Zoomer, H. R., Berg, M. P., de Ruiter, P. C., Verhoef, H. A., Bezemer, T.M. & van der Putten, W. H. (2003). Soil invertebrate fauna enhances grassland succession and diversity. *Nature*, *422*(6933), 711-713.

Dorp, D. V. (1996). *Seed dispersal in agricultural habitats and the restoration of species-rich meadows*, PhD thesis, Wageningen University, 177 pg.

Frey, B., Kremer, J., Rüdt, A., Sciacca, S., Matthies, D., & Lüscher, P. (2009). Compaction of forest soils with heavy logging machinery affects soil bacterial community structure. *European Journal of Soil Biology*, *45*(4), 312-320.

Geissen, V., Wang, S., Oostindie, K., Huerta, E., Zwart, K. B., Smit, A., Ritsema, C.J. & Moore, D. (2013). Effects of topsoil removal as a nature management technique on soil functions. *Catena*, *101*, 50-55.

Hakansson, I., & Voorhees, W. B. (1997). Soil compaction. *Methods for assessment of soil degradation. Boca Raton: Lewis*, 167-179.

Harris, J. (2008). Soil Microbial Communities and Restoration. *Oikos*, *117*, 1833.

Hendriks, M., Mommer, L., Caluwe, H., Smit-Tiekstra, A. E., Putten, W. H., & Kroon, H. (2013). Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. *Journal of Ecology*, *101*(2), 287-297.

Hooper, D. U., Bignell, D. E., Brown, V. K., Brussaard, L., Mark Dangerfield, J., Wall, D. H., Wardle, D.A., Coleman, D.C., Giller, K.E., Lavelle, P., van der Putten, W.H., de Ruiter, P.C., Rusek, J., Silver, W.L., Tiedje, J.M. & Wolters, V. (2000). Interactions between Aboveground

and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks. *Bioscience*, *50*(12), 1049-1061.

Huenneke, L. F., Hamburg, S. P., Koide, R., Mooney, H. A., & Vitousek, P. M. (1990). Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology*, 478-491.

Jansa, J. (2002). *Effect of soil tillage on arbuscular mycorrhizal fungi and on their role in nutrient uptake by crops*. PhD thesis, ETH Zürich.

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I. R., & Frossard, E. (2003). Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications*, *13*(4), 1164-1176.

Jaunatre, R., Buisson, E., & Dutoit, T. (2014). Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, southeast France). *Applied Vegetation Science*, *17*(2), 236-245.

Kardol, P. (2007). *Plant and soil community assembly in secondary succession on ex-arable land: fundamental and applied approaches*. PhD Thesis, Wageningen Universit.

Kardol, P., Bezemer, T.M., & van der Putten, W. H. (2006). Temporal variation in plant–soil feedback controls succession. *Ecology Letters*, *9*(9), 1080-1088.

Kardol, P., Bezemer, T. M., & Van Der Putten, W. H. (2009). Soil Organism and Plant Introductions in Restoration of Species-Rich Grassland Communities. *Restoration Ecology*, *17*(2), 258-269.

Keeney, D.R. & Nelson, D.W. (1982). Nitrogen-inorganic forms. In: Page, A.L., R.H. Miller and D.R. Keeney (eds.) Methods of Soil Analysis. Part 2 – Chemical and microbiological properties. (2nd edition). Agronomy 9:643-698.

Keenleyside, C., Tucker, G., & McConville, A. (2010). Farmland Abandonment in the EU: an Assessment of Trends and Prospects. *Institute for European Environmental Policy, London*.

Kladivko, E. J. (2001). Tillage systems and soil ecology. *Soil and Tillage Research*, *61*(1), 61-76.

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*(6884), 67-70.

Lindemann-Matthies, P., Junge, X., & Matthies, D. (2010). The influence of plant diversity on people's perception and aesthetic appreciation of grassland vegetation. *Biological Conservation*, *143*(1), 195-202.

Neher, D. A. (1999). Soil community composition and ecosystem processes: comparing agricultural ecosystems with natural ecosystems. *Agroforestry Systems*, *45*(1-3), 159-185.

Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939. USDA, Washington, DC.

Read, D. J. (1991). Mycorrhizas in ecosystems. *Experientia*, *47*(4), 376-391.

Read, D. J. (1994). Plant-microbe mutualisms and community structure. In *Biodiversity and ecosystem function* (pp. 181-209). Springer Berlin Heidelberg.

Smit, H. J., Metzger, M. J., & Ewert, F. (2008). Spatial distribution of grassland productivity and land use in Europe. *Agricultural Systems*, *98*(3), 208-219.

SOER Synthesis (2010) The European environment — state and outlook 2010: synthesis. European Environment Agency, Copenhagen.

Török, P., Vida, E., Deák, B., Lengyel, S., & Tóthmérész, B. (2011). Grassland restoration on former croplands in Europe: an assessment of applicability of techniques and costs. *Biodiversity and Conservation*, *20*(11), 2311-2332.

Tsiafouli, M. A., Thébault, E., Sgardelis, S. P., de Ruiter, P. C., van der Putten, W. H., Birkhofer, K., Hemerik, L., de Vries, F. T., Bardgett, R. D., Brady, M. V., Bjornlund, L., Jørgensen, H. B., Christensen, S., Hertefeldt, T. D., Hotes, S., Hol, W.H.G., Frouz, J., Liiri, M., Mortimer, S. R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V. and Hedlund, K. (2014), Intensive agriculture reduces soil biodiversity across Europe. *Global change biology*, *21*(2), 973-985

van de Voorde, T. F., van der Putten, W. H., & Bezemer, T. M. (2012). Soil inoculation method determines the strength of plant–soil interactions. *Soil Biology and Biochemistry*, *55*, 1-6.

van der Heijden, M. G., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A. & Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, *396*(6706), 69-72.

van der Wal, A., van Veen, J. A., Smant, W., Boschker, H. T., Bloem, J., Kardol, P., Van der Putten, W.H. & de Boer, W. (2006). Fungal biomass development in a chronosequence of land abandonment. *Soil Biology and Biochemistry*, *38*(1), 51-60.

Veen, P., Jefferson, R., Smidt, J. D., & Straaten, J. (2009). *Grasslands in Europe of high nature value*. KNNV.

Walker, K. J., Stevens, P. A., Stevens, D. P., Mountford, J. O., Manchester, S. J., & Pywell, R. F. (2004). The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation*, *119*(1), 1-18.

Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Van Der Putten, W. H., & Wall, D. H. (2004). Ecological linkages between aboveground and belowground biota. *Science*, *304*(5677), 1629-1633.

Webb, N. R. (1998). The traditional management of European heathlands. *Journal of Applied Ecology*, *35*(6), 987-990.

Wubs, E. R. J., van der Putten, W. H., & Bezemer, T. M. (2014). Restoration groundwork: testing large-scale soil transplantation to facilitate rapid vegetation development on former arable fields. In *Proceedings of the First Global Soil Biodiversity Conference*, Dijon , France.

APPENDIX 1: INFORMATION ABOUT THE FIELD SITES

Table 5: Information about the location and age of the fields

Type of field	Pair	Field name	Lat Dec. Degrees	Long Dec. Degrees	Abandoned (Year)	Age (in 2015) Year
Arable-field	2	Reemsterakker	52.04606	5.80656	-	-
	1	Reijerscamp	52.01715	5.79041	-	-
	3	Sindehoeve	51.99826	5.75234	-	-
Late grassland	2	Dennekamp	52.02849	5.80170	1982	33
	1	Mosschelse Veld	52.07288	5.73518	1985	30
	3	Wolfhezer Veld	51.99536	5.79057	1988	27
Heathland	3	Doorwerthse Heide	51.99185	5.77512		>100
	1	Mosschelse Veld	52.06861	5.74433		>100
	2	Reemsterheide	52.04106	5.80227		>100

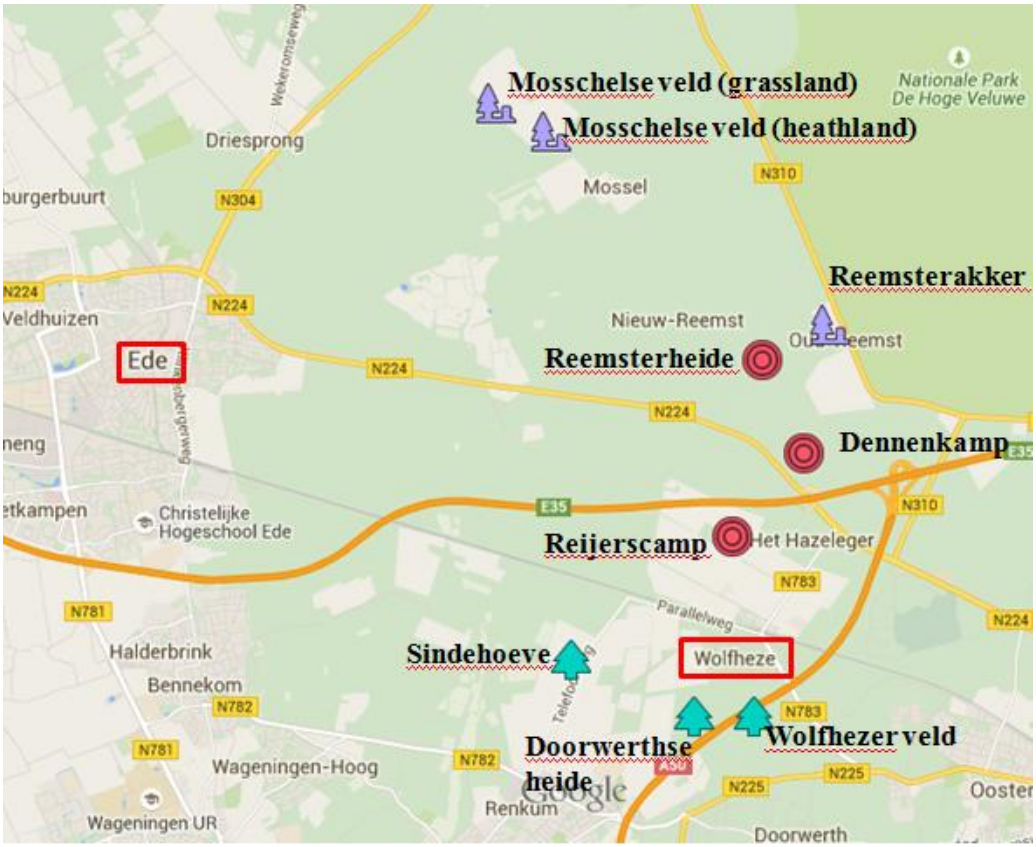


Figure 10: Field sites located on a map, where each symbol represents a couple. The cities of Ede and Wolfheze are highlighted red on the map.