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How performance and recruitment of *Juniperus communis* is effected by bioavailable aluminium.

Investigating the effect of aluminium concentration in the needles of Juniperus communis and the surrounding soil and if aluminium toxicity has an effect on plant performance and how recruitment is influenced by plant performance.

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Abstract

Juniperus communis, is found all over Europe and is considered by the European Council as important habitat in part of heath/grass lands, thus is under protection. However, in recent decades the populations in North West Europe have been under pressure, with declining and aging populations. While main causes such as grazing and changes in land use have been investigated, the effects of acidification and subsequent increase in bio available and phytotoxic aluminium, Al^{3+}, have not been studied in detail. Therefore, two research questions were derived, the first how does aluminium concentrations in the soil and needles relate to the plant performance of *Juniperus communis*? The second, how does plant performance and aluminium concentrations affect the distribution of *Juniperus communis* recruitment?

This study investigated the effects of bioavailable aluminium on the plant performance of *Juniperus communis* and how recruitment is correlating with plant performance measures and bioavailable aluminium. The chemical data and juvenile locations were provided from unpublished datasets, while the plant performance measures, growth, needle age composition and photosynthesis rate were collected in 2019, for the same 40 individuals as from the chemical data. Growth and needle age composition were measured on twigs for each individual and the photosynthesis rate was measured using a “Pulse-Amplitude-Modulation” device, short PAM. The spatial analysis was done based on maps made in QGIS, which used provided and sampled data of 40 junipers.

A Principal Component Analysis showed that the first two principal components account for 65.76% of the variation within the data, thus significantly contribute to the explanation of the variation in plant performance of the *Juniperus communis*. Al ground, pH and needle age composition (NAC) were part of the first principal component (PC), and the second PC consisted of growth and NAC. Photosynthesis rate and NAC 3 show no clear association with either of the two axes. It was found that pH has a negative correlation with aluminium in the soil and photosynthesis rate. A positive correlation between needle aluminium concentrations and pH was found. The rate of photosynthesis correlates negatively with pH and aluminium in the needles and has a positive correlation with aluminium in the ground. Age affects photosynthesis, but not growth or NAC. A higher photosynthesis rate was found in adults. . Furthermore, no decisive spatial pattern was found on how juvenile recruitment is influenced by plant performance and aluminium present in the needles. However, there is a spatial pattern found based on the clustering effect by potential aluminium concentrations within the needles and ground vegetation cover. Hence, it was concluded that no decisive patterns have been found. This suggest that the main influence on the performance is most likely accumulative of the various investigated parameters. In addition a different source might also play an important role in influencing the performance and recruitment of *Juniperus communis*.

Introduction

Juniperus communis, common juniper, is found all over Europe ranging from the most northern regions of Scandinavia to southern Europe, and from the British Isles all the way to the far reaches of the Ural mountain range in Russia (Thomas, El-Barghathi and Polwart, 2007). *J. communis* is considered by the European Council as an important part of calcareous heaths/grass lands and coastal dunes and under the conservation of natural habitats and wild fauna and flora directive (92/43/EEC). Acidic soils with a cover of mosses and dwarf shrubs and low grazing pressure are favourable growing conditions for *J. communis* (Broome *et al.*, 2017). *J. communis* is also considered as an important pioneer species for forest advancement on heathlands and abandoned agricultural land (Arekhi *et al.*, 2018).

The North West European populations of *J. communis* have been found to be under pressure and in a state of decline (Broome *et al.*, 2017). As a result the reasons for the decline have been investigated and it has been found that changes in land use, such as an increase in grazing pressure, fragmentation and aging of junipers have been major causes for the decline (Thomas, El-Barghathi and Polwart, 2007; Legnani *et al.*, 2015; Broome *et al.*, 2017). Furthermore, changes in soil chemistry have added to the decline of *J. communis*. Changes in soil chemistry, were due to atmospheric deposition of nitrogen (Verheyen *et al.*, 2009), deposition of heavy metals (Čeburnis and Steinnes, 2000) and the increasing acidification of the soil due to acid rain and climate change (Rice and Herman, 2012).
Management strategies, such as topsoil removal, liming or tree removal, have yet not resulted in the self-sustaining regeneration and recruitment, which is the goal of the management strategies (Verheyen *et al.*, 2005; Hommel *et al.*, 2013; Broome *et al.*, 2017). In addition, the process of seed dispersal and germination takes multiple years and depends on the seed viability, which decreases as an individual ages. It has been found in other long lived (ca. 200yr) conifer species that the survival of old plants is crucial and that a vital and an age dynamic population has to be kept in order for new recruitment to happen (Verheyen *et al.*, 2005, 2009; Broome *et al.*, 2017).

Older juniper populations might not have the required recruitment success due to the acidification of soils, in order to maintain a sustainable population. While *J. communis* favour acidic growing conditions (Legnani *et al.*, 2015; Broome *et al.*, 2017) acid soils with a pH of 5 or lower also dissolve aluminium. Aluminium is the most abundant metal in the earth's crust and in its solid form not toxic, but transforms into phytotoxic Al^{+3} when dissolved into the soil solution and becomes bioavailable (Roy, Sharma and Talukder, 1988; Kochian, 1995; De Graaf *et al.*, 1997; Matsumoto, 2004; Kochian, Piñeros and Hoekenga, 2005; Ma, 2007; Poschenrieder *et al.*, 2008; Rice and Herman, 2012; Yamamoto, 2018). When a plant takes in Al^{+3}, the aluminium inhibits functions of the roots, resulting in a long term reduction or even a complete stop of the intake of water and nutrients (Roy, Sharma and Talukder, 1988; Kochian, Piñeros and Hoekenga, 2005; Ma, 2007; Guo *et al.*, 2018). The aluminium furthermore inhibits cell division, root growth, and disrupts the structure and function of cell walls, plasma membranes, transduction pathways and Ca homeostasis (Kochian, Piñeros and Hoekenga, 2005; Ma, 2007; Guo *et al.*, 2018; Yamamoto, 2018).However plants have developed methods of dealing with Al^{+3} uptake. For example, by releasing organic anions from the roots and the alkalisation of the rhizosphere (Ma, 2007). Another method is the localisation and concentration of Al^{+3} in the leaves or needles of the plant (Matsumoto, 2004; Poschenrieder *et al.*, 2008). In some cases Al^{+3} can even be beneficial to the plant by increasing the cell membrane integrity and thus resulting in the stimulation of growth, by delaying aging and lignification of the cell (Ma, 2007). However overall is Al^{+3} toxic to the plant and has a negative effect on the plant performance, which can be seen in the decrease of the photosynthesis rate (Guo *et al.*, 2018).

While the effect of aluminium toxicity in plants has been studied (Roy, Sharma and Talukder, 1988; Kochian, 1995; De Graaf *et al.*, 1997; Matsumoto, 2004; Kochian, Piñeros and Hoekenga, 2005; Ma, 2007; Poschenrieder *et al.*, 2008; Rice and Herman, 2012; Yamamoto, 2018), the effect of the uptake by *Juniperus communis* has yet to be investigated in more detail to determine if the Al^{+3} concentration in juniper has an effect on its performance and thus a negative effect on the natural regeneration.

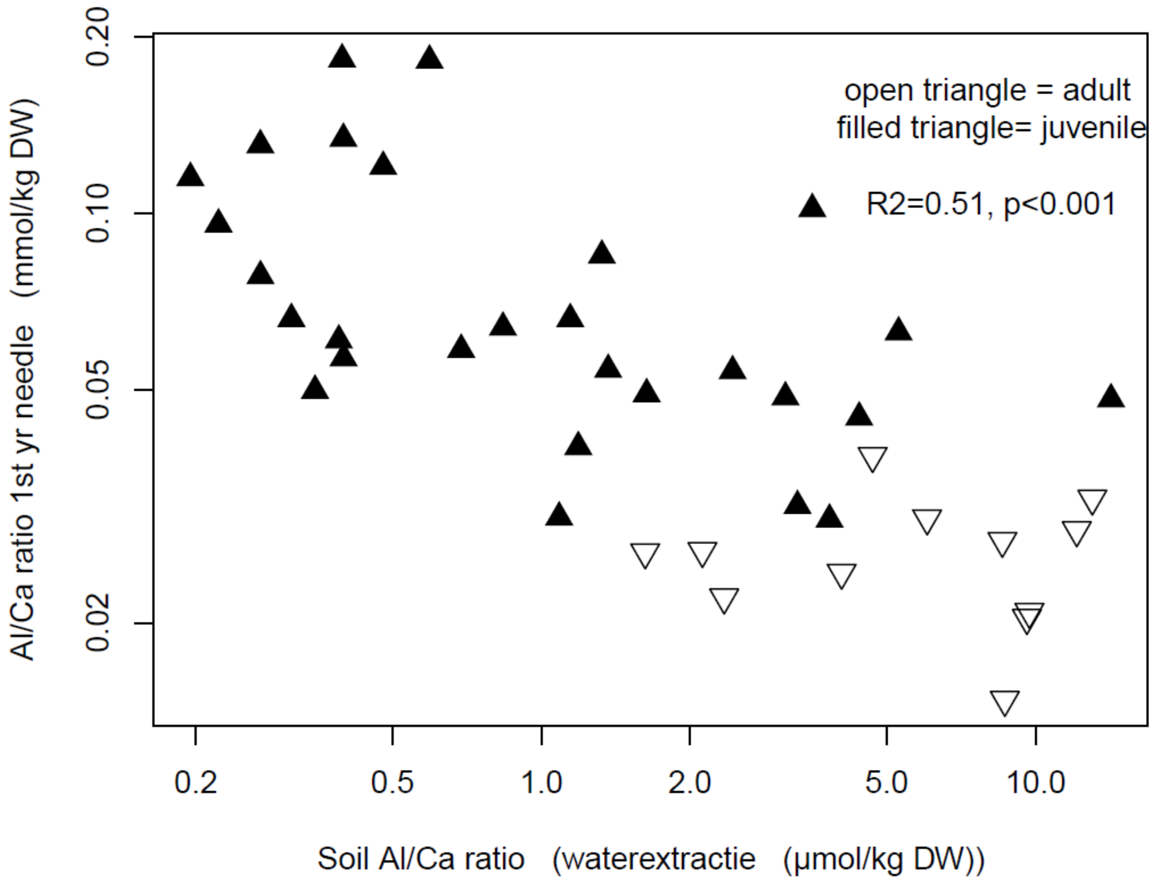

Figure 1 - A scatter plot showing a highly significant negative correlation between Al/Ca ration in juniper needles per μmol/kg DW and Al/Ca ration in the soil, distinguishing between adult and juvenile individuals (Veldhuis, unpublished).

A previous study (Veldhuis, unpublished) found a significant negative correlation between aluminium/calcium concentrations in the needles (Veldhuis, unpublished). In addition, they found that adult plant needles contain lower aluminium/calcium concentrations than juvenile plants (Fig. 1). This was an unexpected finding, as it was expected that older individuals would have absorbed more aluminium from the soil, over their life time. However, the effects of the aluminium concentration on Juniper performance are so far unclear. Therefore, this study was carried out to investigate the effect of aluminium concentrations on *J. communis*. Another goal of this study was to find out how juvenile recruitment is influenced by aluminium concentrations and plant performance measures. Furthermore, the aim of this study was to assess how aluminium concentrations in *Juniperus communis* needles and the surrounding soil affect the plant performance, growth, needle age composition and photosynthesis rate as measure of plant performance and age group.

From this two research questions were derived, the first how does aluminium concentrations in the soil and needles relate to the plant performance of *Juniperus* and the second how does plant performance and aluminium concentrations relate to the distribution of *Juniperus communis*. It was expected to find that aluminium concentrations within the needles and soil will have a negative effect on plant performance, to a different extend based on plant age, adult and juvenile.

Methods

Study Site

The study area Drouwenerzand, Figure 2, is a dry heathland located near Drouwen, Drenthe a province in northern Netherlands. Drouwenerzand is a Nature area managed by het Drentse Landschap and is open to the public with designated walking paths through the area. The annual average precipitation is around 810mm, with an even spread around the year, with slightly lower rates of precipitation during the spring months compared to the rest of the year. The annual average daily mean temperature is around 9°C, which can reach above 20°C during the summer and around 0°C during the winter months (KNMI, 2016).

This area has a larger population of *J. communis* and is predominantly covered with *Caluna vulgaris* heathlands, and scattered pine (*Pinus sylvestris*) and oak (*Quercus sp.*) trees. The soil of the area is of poor quality with low nutrient levels and predominantly sand to sandy top soils. There is a small open dune system in the west of Drouwenerzand, see Figure 2.

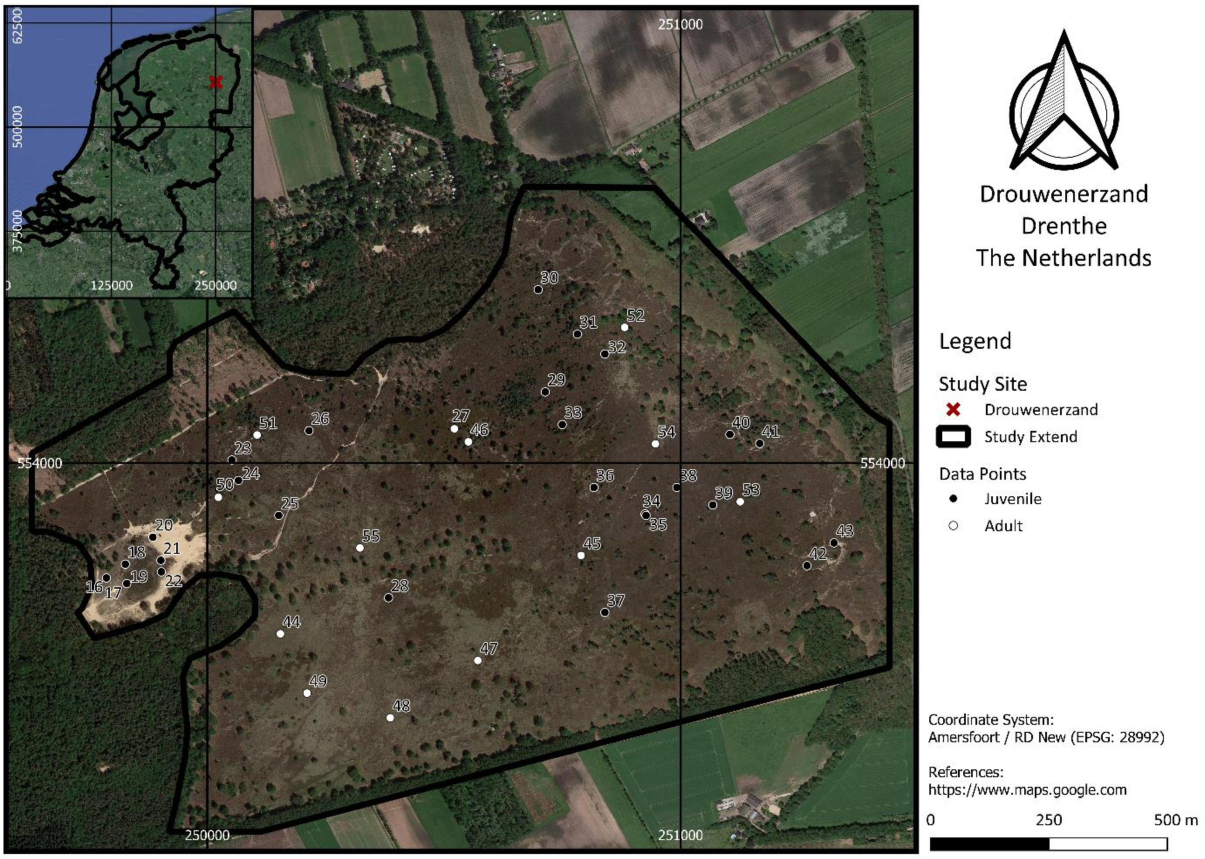

Figure 2 – Map of the study site in Drouwenerzand, Drenthe the Netherlands. The inset map shows the location of the study site in the context of the Netherlands. The main map shows the study site in Drouwenerzand with the 40 sampled *Juniperus communis*, showing which juniper was adult or juvenile with the sample number.

Sampling methods

Original dataset

An unpublished dataset, from Veldhuis, consisting of 40 individuals was provided, which consisted of 12 adults and 28 juvenile *J. communis*. Furthermore, it contained a series of chemical data from soil and needle analysis, which were from the 40 sampled individuals. From the unpublished dataset aluminium concentration in the first year needles and in the top 25cm of the soil and pH were used in this study. These junipers were sampled in 2017. Al concentration in the first year needles, Al concentration in the top 25cm of the soil and soil pH were used from the provided dataset.

Additional measurements

All 40 junipers were revisited in 2019 for additional measurements on growth and needle age composition. Last year's growth measure was used, since the junipers were already in their growing season, and referred to as growth. The procedure for the juveniles was that 5 twigs were chosen at random and last year's growth on the main twig was measured. For adults, 5 twigs in each cardinal direction were chosen at random, so a total of 20 twigs were sampled. If an area of an individual was partially obstructed by another juniper then the closest branch to the cardinal direction was chosen. Furthermore, if an individual was heavily grazed and no twigs were present with last year's growth fully intact then this individual was to be noted down as NA, but still included in the analysis. This was the case for 17 juvenile individuals.

For the needle age composition the same 40 individuals and twigs were used. Needle age composition is estimated as closely as possible, by looking at the twig and each year's growth section. Juvenile junipers were sampled of the last 3 years growth seasons. This was done because the branches, of juvenile individuals, were limited in length and after 3 years the amount of present needles were most of the time 0%. For adults, the growth sections of the last 5 years were measured, following the same measuring procedure as the juveniles. The estimation was based on the visible amount of needles still present and how the next section compared to the previous section. With that points were needles have been in the past could have been became noticeable and give an estimate of how many needles were still present.

To measure the photosynthesis rate of the 40 individuals a "Pulse-Amplitude-Modulation" device, short PAM, was used in the field (Baker and Oxborough, 2004). For this study, the model IMAG-CM with an IMAG-K2 were used. The measuring light was set to an intensity of 2 and a frequency 1. The activation light was set to an intensity of 13 with a width of 0 seconds. The mini image correction was selected. The saturation pulse was set to an intensity of 9 with a number of 1 and an interval of 30 seconds. The slow induction was set to a delay of 40 seconds, clock to 20 seconds and a duration of 315 seconds. These settings were determined by trial measurements of *J. communis* samplings that were grown in a greenhouse.
In the field, the PAM was fixed into position with a tripod. On the sampled juniper a south facing twig was chosen at random. This twig was then fixed into position in the PAM, as seen in Figure 3A, and the lens of the PAM was adjusted so that the twig was in focus. On the laptop that was connected to the PAM the software ImagingWin v2.41a was used to perform the measurements. On the sampled twig, 5 zones of roughly the same area were highlighted as the measurement area using the software. Then the twig and PAM was covered, as seen in Figure. 3B, and let to be dark adapted for 5min after which the measuring programme was run. This was then done for all 40 junipers. From the measurements Fv/Fm was calculated and henceforth referred to as photosynthesis rate. Fv is the variable chlorophyll fluorescence when non-photochemical processes are at its lowest, when the needle is dark adapted. This is then divided by the Fm, which is the maximum fluorescence at the

highest level, when the plant is exposed to light. The resulting Fv/Fm then give a value for the quantum efficiency of photosynthesis of a plant, i.e. the potential photosynthesis rate of a plant. A value for Fv/Fm around 0.8 to 0.7 is considered to be a good value, i.e. that a plant is not stressed and has a high rate of photosynthesis (Cavender-Bares and A. Bazzaz, 2004).

Figure 3 – A, The PAM fixed by the tripod and a twig fixed within the measurement area of the device. B, The covered PAM for the dark adjustment of the juniper twig. The PAM was covered in multiple layers of black bags.

Part of the analysis is done in QGIS 3.4, with the aim of investigating how plant performance measures and aluminium in the needles affect the recruitment distribution of *Juniperus communis*. The sampled and provided data for the 40 junipers were imported into QGIS and there further analysis was run for the whole study site. Then another dataset from 2012 was provided, which contained 295 sampled juvenile junipers from the same study area, with positional data, this dataset was sampled as part of an unpublished study in 2012. The methodology for recording these 295 individuals was that only junipers with a height less than 1m were recorded, since junipers larger than 1m were considered adults. These 295 junipers were then added to the study site map in QGIS.

Data analysis

Two research questions were formulated. Firstly, how does aluminium concentrations in the soil and needles relate to the plant performance of *Juniperus communis*? Secondly, how does plant performance and aluminium concentrations affect the distribution of *Juniperus communis* recruitment? Based on these two research questions four hypothesis were formulated: 1) There is a relationship between plant performance measures and chemical parameters, 2) There is a significant correlation between plant performance measures and aluminium concentrations, 3) There is a significant difference in mean plant performance measures, between plant age groups, and 4) There is a pattern of the spatial distribution (clustering) of juvenile recruitment depending on growth, needle age composition, photosynthesis rate and aluminium concentration in the needles, based on already established junipers. These four null hypothesis have been made: There is no relationship between plant performance measures and aluminium concentrations. There is no significant correlation between plant performance measures and chemical parameters. There is no significant difference in mean plant performance measures, growth, needle age composition and photosynthesis rate between aluminium concentration within needles and plant age group. There is no pattern of the spatial distribution of juvenile recruitment depending on growth, needle age composition,

photosynthesis rate and aluminium concentration in the needles, based on already established junipers.
First, the data of the 40 sampled junipers containing growth rate, needle age composition and photosynthesis rate, and the existing database with aluminium in needles and soil and soil pH, were screened for normality using a Shapiro-Wilk test (Dytham, 2011). However the ground and needle aluminium concentrations were not normally distributed, even after a log10 transformation. No further attempts of transformation were undertaken to simplify analysis and further interpretation and a normal distribution was not a necessity in the further analysis process.

To find out the relationship between rate of photosynthesis, growth rate and needle age composition (plant performance measures), and aluminium in the top soil, aluminium in the needles and pH in the soil (chemical parameters) a Principal Component Analysis (PCA) was used. It was chosen to show the correlation between the different plant performance measures and chemical parameters. A normal distribution of the used data is recommended, but not necessary if it is assumed that the data conforms to a linear trend. In this case a non parametric correlation was used to answer the second hypothesis and the results were also compared to the results of the PCA, in order to validate the findings (Dytham, 2011).
Since data was non-normally distributed, Spearman's Rank Correlations were used to test how plant performance measures correlated with the chemical parameters (Dytham, 2011).
In order to investigate the effects on growth rate, needle age composition and photosynthesis rate by age group one way ANOVAs were carried out. First the data was screened if it conforms to an equal variance, by using a Levene's Test. The aluminium in the needles data was not homogeneously distributed, and thus was transformed, the data was transformed by the power of two. The one way ANOVA model had either growth, NAC or photosynthesis rate as the response variable and age (juvenile or adult) as the predictor variable. After the ANOVA model was run the residuals were tested for normality using a Shapiro-Wilk test (Dytham, 2011).

Lastly, the spatial analysis was done in QGIS 3.4. The plant performance data and chemical parameters were interpolated for the study site using the Inverse Distance Weighted (IDW), technique. The IDW method was chosen since the data was not uniformly distributed across the study site and with the IDW method the clustering of sample points and the distance was taken into account and more accurate interpolations for this distribution would be produced (Watson and Philip, 1985).
The statistical analysis was done in R and preliminary data screening was done in Minitab 17, and Choosing and Using Statistics – A Biologist's Guide, by Calvin Dytham (Third Edition, 2011), was used to determine which statistical test is used.

Results

PCA

In Figure 4 the results of the PCA of plant performance measures and chemical parameters are shown. Adult and juvenile individuals show a clear separation, in Figure 4, which is also shown in Figure 1. Three principal components have eigenvalues greater than one, which indicates that there is a third dimension to consider. The first two principal components account for 65.76% of the variation within the data, thus significant contribute to the explanation of the variation in plant performance of the *Juniperus communis*. Al ground, pH and NAC 3 were part of the first principal component (PC), and the second PC consisted of growth and needle age composition of the 3rd year. Photosynthesis rate and NAC 3 show no clear association with either of the two axes. Photosynthesis rate, has a moderate loading plots for PC1 a score of 0.44, compared to -0.38 in PC2. While NAC 3 has PC1 score of 0.13, and a PC2 score of -0.14.

pH and aluminium in the needles, as seen in Figure 4, correlate with a PC1 score of -0.56 and -0.44 respectively and are mostly explained by the first principal component. Al ground is also mostly explained by the first principal component, with a score of 0.52, and is negatively correlated to Al ground and pH. NAC 3 and photosynthesis rate strongly correlate. A correlation of NAC 3 and photosynthesis with Al Ground is suggested, as well as a negative correlation between pH and Al needles and NAC 3 and photosynthesis rate. Growth does not correlate with any of the other parameters and is mostly explained by the second principal component, with a score of 0.89.

In line with the first hypothesis, there is a relationship between plant performance measures and chemical parameters, except for growth rate that does not well correlate with the other parameters.

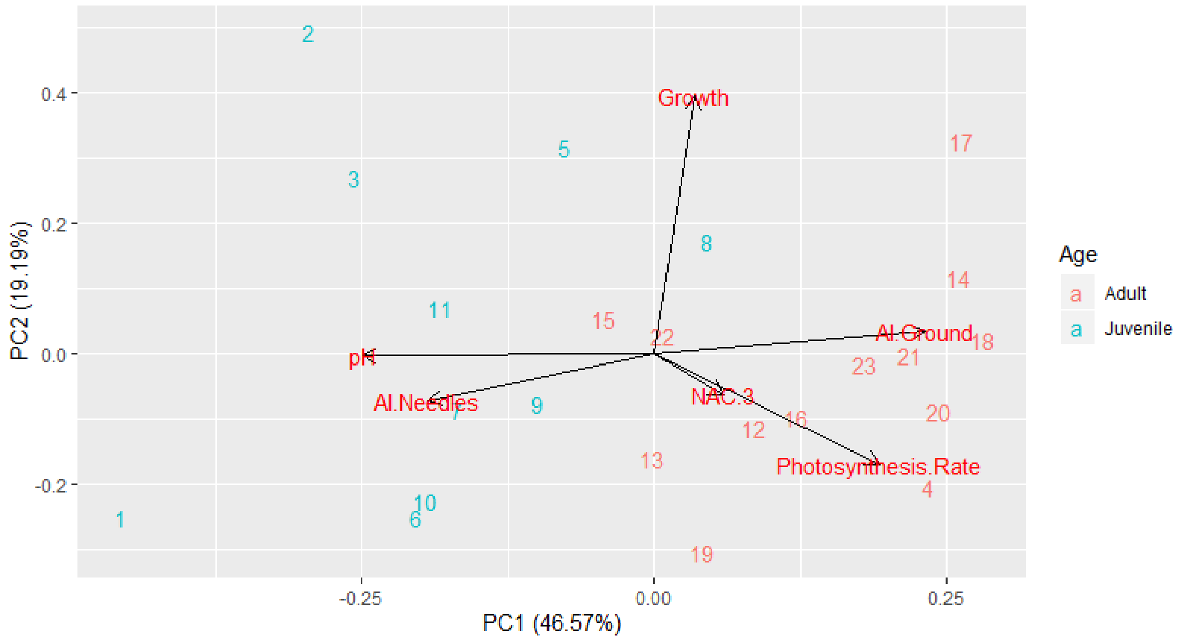

Figure 4 - Site Plot showing the Principal Component Analysis (PCA) results of the similarity between juniper age, with the loading plot showing the eigenvectors of the plant performance measures (Growth – Growth rate, NAC.3 – Needle age composition, Photosynthesis rate) and chemical parameters (pH – soil pH, Al.Needles – Aluminium in 1 year old needles and Al.Ground – Aluminium in the top 25cm of the soil) present in both age groups, showing the relationship between each factor.

Correlations between variables

In Table 1, the Spearman's Rank Correlation Coefficients are shown. Al ground is significantly positively correlated with photosynthesis rate. pH is highly significantly negatively correlated with photosynthesis rate and Al in the soil. pH is highly significantly positively correlated with Al in the needles. Al in the needles is highly significantly negatively correlated with photosynthesis rate and Al in the soil. Plant performance measures do not correlate among each other. Photosynthesis rate is the only plant performance measure that correlates with the chemical parameters. All the chemical parameters correlate among each other. Several parameters are close to statistical significance, which might suggest a possible correlation. Al in the needles and needle age composition also suggests a negative correlation, with a P value close to 0.05 (a trend).

The second hypothesis, there is a significant correlation between plant performance measures and aluminium concentrations, is partially true. There is a correlation among chemical parameters, but no correlation among plant performance measures, and only a correlation between photosynthesis rate and chemical parameters. Also, some correlations between plant performance measures and chemical parameters might be possible.

Table 1 – Correlations Matrix of plant performance measures (Growth, needle age composition 3rd year needles – NAC 3 and Photosynthesis rate) and chemical parameters (soil pH, aluminium ground and aluminium needles). (Spearman's Rank Correlation Coefficient, df = 38). Significance values: * P < 0.05, ** P < 0.01, *** P < 0.001

	Growth	NAC 3	Photosynthesis Rate	pH	Al Ground	Al Needles
Growth	----					
NAC 3	-0.04	---				
Photosynthesis Rate	-0.14	-0.14	---			
pH	-0.20	-0.27	-0.52***	---		
Al Ground	0.29	0.14	0.39*	-0.81***	---	
Al Needles	-0.19	-0.37	-0.47**	0.66***	-0.44**	---

ANOVA

There was no significant difference in mean plant performance measures between young and old individuals. In Table 2 the ANOVA Pr values and P values for the normality test are shown. Growth has a high Pr value, 0.88, which shows that there is no significant difference in mean growth and age, with normally distributed residuals. NAC has a lower Pr value, 0.27, and thus has no significant difference in mean NAC and age, and the residuals also conform to a normal distribution. Lastly, photosynthesis rate does show a significant difference in mean photosynthesis and age, Pr value of 0.01. The standard error is very small. And showed that adults have a higher photosynthesis rate than juveniles. However the residuals do not conform to a normal distribution.

The third hypothesis, there is a significant difference in mean plant performance measures, growth, needle age composition and photosynthesis rate between plant age groups, is mostly rejected. There is no significant difference in mean growth and age. There is also no significant difference in mean needle age composition and age. The only significant difference is between mean photosynthesis rate and age, with a higher photosynthesis in adults.

Table 2 – Results of the ANOVA models. Showing how plant performance measures (Growth, needle age composition 3rd year needles – NAC 3 and Photosynthesis rate) were influenced by the plant age (ANOVA, df = 38).Significance levels: ** Pr < 0.01, * P <0.05, *** Pr <0.05

Coefficient	Std. Error	Pr Value	F value	Normality P Value	Levene Pr Value
Growth ~ Age	0.57	0.88	0.025	0.11	0.25***
NAC 3 ~ Age	10.83	0.27	1.263	0.26	0.51***
Photosynthesis Rate ~ Age	0.02	0.01**	30.74	0.01*	0.29***

Spatial analysis

Relationship juniper juvenile density and Al in needles

In Figure 5, a distribution of juniper juveniles sampled in 2012 can be seen, with a division between east and west, with a few in the middle mostly in the north of the study area. In Figure 5 Map 1, there is a strong clustering of juveniles in the west most part of the area, which also has a high potential concentrations of Al in the needles (18µmol/kg). Figure 6 Map 1, shows a clear picture of the high concentration of juveniles in the west. While there is a large number of juveniles in the east, there they are more widely distributed over the eastern extend and would have less Al in the needles (3 – 5 µmol/kg). Juvenile density seems to depend on the amount of potential Al in the needles present. Higher potential concentration areas show a denser clustering than areas with lower concentrations of aluminium in the needles.

Relationship juniper juvenile density and growth rate

Average growth rate of the juveniles in the area is 4 cm (±1.3cm), as seen in Figure 5 Map 2. There are two areas with larger growth rates, one in the south west and the other in the north. The northern growth area has a number of juveniles establishing there. Comparing the aluminium base map with the growth base map, Figure 5 Map 1 and 2 , it can be seen that areas with high aluminium concentration in the needles there is a lower growth rate and vice versa.

Relationship juniper juvenile density and needle age composition

In Figure 5 Map 3, the needle age composition of 3rd year needles is shown. There are two distinct areas with high needle age compositions, the first is in the south and the second is in the eastern most side. In both areas few to no juveniles are established. Most juveniles are established in areas with low needle age composition, surrounding the lowest areas on the map, such as in the north and between the two high composition areas.

Relationship juniper juvenile density and photosynthesis rate

The east and west parts of the study site have the lowest photosynthesis rates, as seen in Figure 5 Map 4, with Fv/Fm values lower than 0.5. While in the eastern area with low Fv/Fm only small number of juveniles established the opposite can be seen in the western area, where the dens clustering of juveniles can be observed. However, most of the surface has a value between 0.6 and 0.7 Fv/Fm.
Comparing the needle age composition and photosynthesis rate base maps, it can be seen that the eastern area has low photosynthesis rates, but a high needle age composition. Which is not the case in the western area, where in the area with low photosynthesis rates the needle age composition is also low. Much like the pattern of dense cluster of juveniles in high aluminium area (Fig. 6 Map 1), a similar pattern of the dense cluster in a low photosynthesis area can be seen (Fig. 6 Map 2). Comparing the aluminium in the needles with the photosynthesis base map, the eastern most area also has one of the lower aluminium contraptions, but unlike other areas with low aluminium concentrations such as the south west, the Fv/Fm values are very low, below 0.5 Fv/Fm. Most consistent overlap seems between the growth and photosynthesis rates, where slightly above average growth, 4cm, cover the same area as higher Fv/Fm, 0.7.

The fourth hypothesis, there is a pattern of the spatial distribution of juvenile recruitment depending on growth, needle age composition, photosynthesis rate and aluminium concentration in the needles, based on already established junipers, is not supported by the data. There is no clear pattern of juvenile distribution. The most apparent pattern is when comparing growth and photosynthesis rates, where slightly above Fv/Fm values seem to be in the same areas as average growth rates. As for distribution of the juveniles no clear pattern based on the plant performance measures can be extracted.

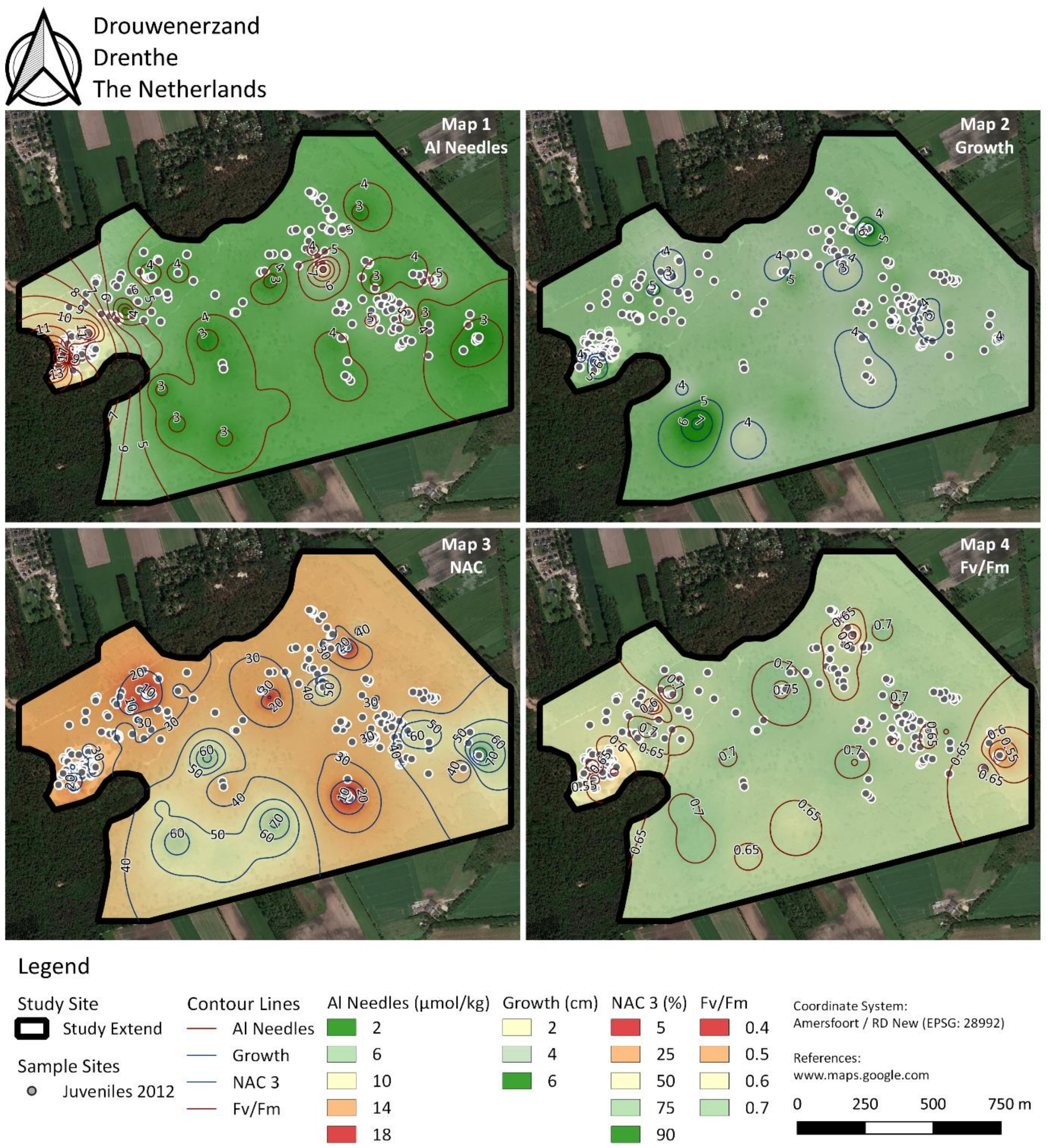

Figure 5 – Maps show the study site in Drouwenerzand, Drenthe Netherlands, with the 295 sampled juvenile *Juniperus communis* over interpolated maps of the plant performance measures. Map 1 shows the aluminium concentrations in the needles with the juvenile junipers. A low value of aluminium in the needles is better for the plant and thus represented in a green colour. Map 2 shows the interpolated results of the growth over one year. Map 3 shows the needle age composition of the 3rd year of the juvenile junipers. Map 4 shows the interpolated results of the photosynthesis rate of a juniper. For the Maps 2 – 3 values that are high are represented in a green colour as a higher value is better for the plant.

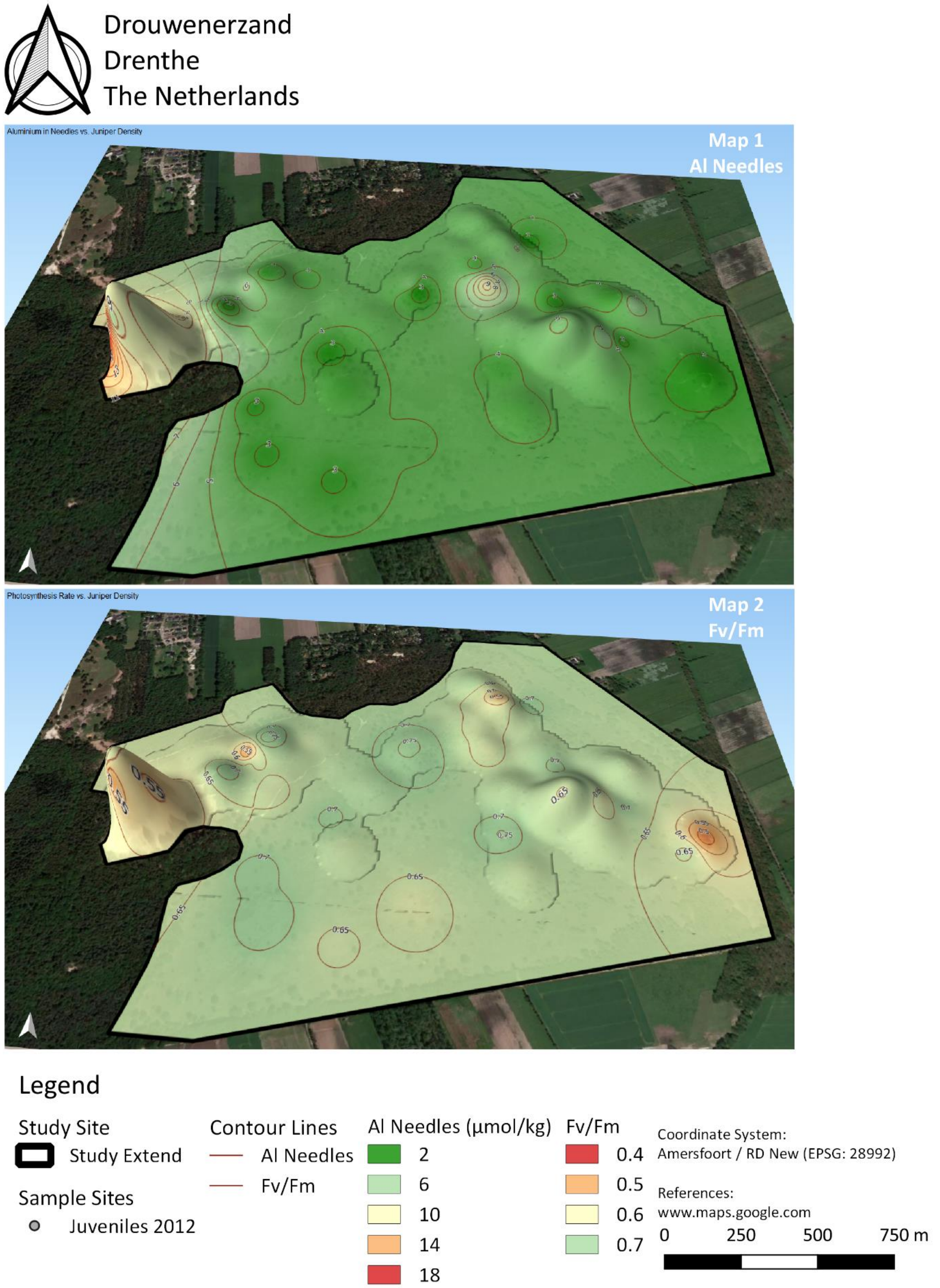

Figure 6 – Maps show the study site in Drouwenerzand, Drenthe Netherlands, same as in Figure 5. Peaks show the density of the 295 Juveniles sampled in 2012, higher peak means more individuals closer together, clustering. Map 1 shows the aluminium concentrations in the needles with the juvenile junipers. Map 2 shows the interpolated results of the photosynthesis rate of a juniper.

Discussion

In this study, it was investigated how aluminium concentrations within soil and needles and soil pH (chemical parameters) relate to the growth rate, needle age composition and photosynthesis rate (plant performance measures). It was found that the chemical parameters correlated among each other and that photosynthesis rate correlated with the chemical parameters (Table 1), which suggests that the rate of photosynthesis is lower with plants that absorbed more aluminium or are on soil with lower aluminium concentration and perform less well. Also, it was found that denser clustering of juveniles occurred at areas with higher potential Al in the needles and that juvenile recruitment occurred in areas with denser plant coverage, like grasses and *Caluna vulgaris* (Figure 1 and 5). This probably indicates that juvenile distribution is affected by aluminium concentrations and that ground vegetation cover matters.

Acidification of the soil, by for example SO_2 pollution, is considered as a cause for the loss of *J. communis* (Fitter and Jennings, 1975). However the exact pinpointing of the source of acidification is difficult, due to the wide range of sources, for example from vegetation changes (Anita and Madhoolika, 2008). Bioavailable aluminium, Al^{+3}, is phytotoxic, which is well known by now (De Graaf *et al.*, 1997; Ma, 2007; Yamamoto, 2018). Plants have developed various methods of dealing with inorganic pollutants such as aluminium. A physical defence is to store the pollutant in a leave or needle and to sacrifice it (Poschenrieder *et al.*, 2008). Chemical defences are also used such alkalisation of the rhizosphere and the production of organic acidic anions within the roots (Ma, 2007). In Figure 5 it can be seen that most juveniles are more dispersed in areas with low needle age composition (Figure 5, Map 3). Those areas also show low amounts of aluminium present in the needles (Figure 5, Map 1), this shows that the plants are deploying such a defence mechanism. The most likely mechanism would that of the sacrificial needle.

With a pH of 5 or lower aluminium turns bioavailable and as a result more aluminium is taken up by the plant (De Graaf *et al.*, 1997; Ma, 2007; Rice and Herman, 2012; Guo *et al.*, 2018; Yamamoto, 2018), with lower levels of aluminium remaining in the soil This was found in the PCA, in Figure 4, where pH and aluminium in the needles show a positive correlation, and pH is negatively correlated with aluminium in the soil. Furthermore, it can be seen, in Figure 4, that adults tend to be associated with aluminium in the soil and juveniles with aluminium in the needles. A study by Matsumoto (2004), found that over time the uptake of aluminium is reduced, which might be one of the factors explaining this observation. Al replaces the Cl in the cells and over time less acidic conditions slow down the uptake of Al by the plant (Matsumoto, 2004).

The PCA (Figure 4) shows that growth is not correlated with anything, which suggests that growth is not a conclusive parameter to determine plant performance. From Table 1 it can be seen that photosynthesis rate is a good predictor to see how aluminium affects the performance of a plant. Photosynthesis rate negatively correlates with both soil pH and aluminium in the needles. A positive correlation is seen with aluminium in the soil. This trend can be explained by, if the soil pH is low more aluminium is bioavailable and taken up by the plant and vice versa, and as a result of higher concentrations of aluminium in the plant the rate of photosynthesis decreases (Guo *et al.*, 2018).

Aluminium can be concentrated within the plant to limit the effects on the plant performance. The most common places are leaves or needle cell walls or leave vacuoles (Matsumoto, 2004; Poschenrieder *et al.*, 2008). While this is not entirely supported by the collected data, the spatial distribution of juveniles does show that plants in low needle age composition areas also have lower amounts of aluminium in the needles present. This shows that there is the possibility that the mechanism of sacrificial needles is most likely used by the *J. communis*. As a negative correlation between needle age composition and aluminium in the needles is suggested, as seen in Table 1, which showed a non-significant trend, but the P value for the Spearman's Rank Correlation Coefficient was close to being significant and showing a negative trend. Furthermore, it is questionable whether size of an individual should be used as an indicator for performance (Zeidler, Banaš and Ženatá, 2009), which is supported by result that none of the tested parameters correlated with growth of an individual (Table 1). In addition, there is currently no consensus at what point aluminium toxicity starts to affect the cellular processes (Kochian, Piñeros and Hoekenga, 2005), thus it is difficult to say when an individual exposed to high levels of bioavailable aluminium has its performance effected.

It has been shown that both temperature and nitrogen deposition have a negative direct and indirect influence on the plant performance of junipers, with a negative effect on seed viability (Verheyen *et al.*, 2009; Ward, 2010; Gruwez *et al.*, 2014). *Juniperus communis* prefers cooler and wetter conditions, especially as a juvenile (Thomas, El-Barghathi and Polwart, 2007). Adults on the other hand are more drought resistant and show better signs of recovery after drought conditions (Herrero and Zamora, 2014). Junipers are especially sensitive to droughts during the summer months (García *et al.*, 1999). With the effect of climate on *J. communis* and cooler and wetter conditions being preferred warmer conditions do lead to more growth (Shetti *et al.*, 2018). Sex also has an influence on how well an individual can deal with stress, since there is a difference in energy and nutrient use between males and females, with females needing more nutrients and energy for seed production (Marion and Houle, 1996). As a result females may be more sensitive to climatic changes (Rozas, DeSoto and Olano, 2009). Furthermore male junipers can survive longer in poor health, growing older than their female counter parts (Ward, 2007). Hence, given the sandy soils in Drouwenerzand, juvenile establishment may be more difficult.

Regeneration of populations is considered to be one of the greatest long term threats for the *J. communis* populations (Clifton, Ward and Ranner, 1997). With ageing populations, a number of problems arise, such as reduction of seed viability and quality of pollen (García *et al.*, 1999; Verheyen *et al.*, 2009; Broome *et al.*, 2017). For older junipers, the increase in browning of the crown can be used as an indicator of their performance, but not size (Zeidler, Banaš and Ženatá, 2009). With changes in weather patterns heat can influence the quality of pollen reducing the fertility of populations (Verheyen *et al.*, 2009). Also the viability of seeds is age dependent, with older seeds being less viable for reproduction (Verheyen *et al.*, 2009; Ward, 2010; Broome *et al.*, 2017). Furthermore, *J. communis* is a male dominated species, which can lead in aging populations to the cases that viable seeds being present is too low (Thomas, El-Barghathi and Polwart, 2007). In addition, seeds are also often predated on by insects, leaving a large proportion of the seeds empty (Tylkowski, 2009) and it is shown that bigger junipers host more insects (Zeidler, Banaš and Ženatá, 2009). Photosynthesis is influenced by age, as seen in Table 2, with higher photosynthesis rates for adults compared to juveniles. A possible reason for this could be that juveniles have more aluminium in the needles Figure 5 Map 1. This would be supported by fact that juveniles are more sensitive to soil chemistry (Allegrezza *et al.*, 2016).

In this study it has been found that most of the juveniles seem to be established in areas that are covered by *Caluna vulgaris* (Figure. 2 and 5), which is in line with what other studies have found. These studies show that site condition also plays a big role in the regeneration of *J. communis* (Fitter and Jennings, 1975; Clifton, Ward and Ranner, 1997; García, 2001; Broome *et al.*, 2017; Ward and Shellswell, 2017). In general, *J. communis* needs open and disturbed areas for regeneration and for juveniles to establish (Fitter and Jennings, 1975; Clifton, Ward and Ranner, 1997; Broome *et al.*, 2017; Ward and Shellswell, 2017). The site also has to be slightly acidic and wet, in order to not be strongly influenced by drought conditions (García *et al.*, 1999; Gruwez *et al.*, 2014; Broome *et al.*, 2017). It has been observed that juveniles grow near rocks or under other junipers, to lessen the effects of drought (García, 2001). A similar effect is an advantage for established juveniles, which is that denser vegetation provides moister growing conditions (Rosen, 1988). Another reason for juveniles to establish near rocks is that birds, which feed on the seeds, use those rocks a perch and excrete the seeds around those rocks, but is considered to be not an important factor for dispersal (Houle and Duchesne, 1999; Verheyen *et al.*, 2009).

In Figure 2, open areas can be seen in the south and west of the study site, other areas of the site are covered in grasses and are relatively open. However, in Figure 6, it can be seen that almost no juveniles have established in the open southern area, which deviates from other studies that focus on regeneration. It has been found that natural regeneration usually occurs on bare soil or short vegetation cover (Fitter and Jennings, 1975), part of the lack of juveniles might be due to the presence of grazers, in this case sheep, which roam this area in a high frequency. The negative effect of grazing on the establishment of juveniles, has been shown extensively (Fitter and Jennings, 1975; Clifton, Ward and Ranner, 1997; Thomas, El-Barghathi and Polwart, 2007; Verheyen *et al.*, 2009; Zeidler, Banaš and Ženatá, 2009; Broome *et al.*, 2017; Ward and Shellswell, 2017). Fitter and Jennings (1975), suggest that grazing should be done gradually and individuals to be fenced off. The gradual grazing would reduce the stress on juveniles and result in a better regeneration and the higher mortality during winter months by grazing and trampling could be prevented. In habitats where there is no disturbances however, *J. communis* is out competed by other plants (Fitter and Jennings, 1975; Zeidler, Banaš and Ženatá, 2009; Ward and Shellswell, 2017). This happened in the primeval forests of the Ukraine Carpathians (Šebesta *et al.*, 2011). Old agricultural practices did promote natural regeneration, by using rotational low intensity grazing (Ward and Shellswell, 2017). Another, management strategy could be to grow juniper saplings under controlled conditions, by using cutting of healthy individuals and then planting them out (Tylkowski, 2009; Ward and Shellswell, 2017).

Conclusion

It has been found in this research that aluminium in both soil and needles have an effect on the performance of *Juniperus communis*. There is a negative correlation between aluminium concentrations in the soil and pH and a positive correlation between aluminium concentrations in the needles and pH. Also there is a negative correlation between the photosynthesis rate and pH and aluminium in the needles. The positive correlation between the photosynthesis rate and aluminium in the soil, showed that there is more bio available aluminium in more acidic soils and is not taken up by the plant. Furthermore the negative correlation, between photosynthesis and aluminium concentrations in the needles shows that the plant is taking up the bioavailable aluminium. When this is the case the aluminium in the ground is then lower and in the needles higher. Junipers seem to deploy the method of creating sacrificial needles to get rid of excess aluminium, as the areas with low needle age composition and high Al in the needles overlap and as an almost negative correlation between needle age composition and aluminium in the needles was found. Furthermore, it is found that there is a significant difference in mean photosynthesis, with adults having higher photosynthesis rates and as a result being less influenced by the aluminium. Growth and needle age composition on the other hand do not show a significant mean difference with age. Lastly, there was no clear overall spatial pattern visible in the distribution of juveniles when plotted against plant performance measures and aluminium present in the needles. There is a pattern of distribution based on ground vegetation cover. Also there is denser clustering when there is a higher potential aluminium concentrations within the needles. In conclusion, aluminium does have some effect on *Juniperus communis*. However, no decisive patterns have been found, which suggest that the main influence on the performance is most likely accumulative of the various investigated parameters. In addition a different source might also play an important role in influencing the performance and recruitment of *Juniperus communis*.

The sample size was a limiting factor and a more even split between adults and juveniles would have been more ideal. As well, as a more even physical distribution of samples, would have made the spatial analysis more conclusive. Furthermore, to flash out the study parameters vegetation cover, grazer presence and activity and climactic data should be added for a possible future study to give a more holistic picture.

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