Plant Soil
https://doi.org/10.1007/s11104-024-06857-7

RESEARCH ARTICLE

How nitrogen deposition hampers common juniper regeneration in heathlands

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Received: 27 August 2023 / Accepted: 15 July 2024

Abstract

Aims Common Juniper *(Juniper communis)* populations in western Europe are rapidly declining due to a lack of regeneration, which has been linked to nitrogen (N) deposition. However, the mechanisms by which N deposition affects juniper are not clear. Nitrogen deposition can lead to N eutrophication and soil acidification, which cause nutrient leaching and metal mobilisation with potential negative impacts on juniper regeneration. We investigated associations among soil element concentration (soil [E]), needle element concentration (needle [E]) and plant performance in terms of height growth and viable seed production.

Methods We sampled adult females and juveniles across 6 natural heathland areas in the Netherlands and Belgium, and applied linear mixed models for eleven elements to test associations among plant performance, soil [E], and needle [E].

Results Soil [E] was reflected in needle [E], which subsequently was associated with juniper growth. However, direct associations between soil [E] and growth were absent. Seed viability was positively associated with soil Mg concentration, marginally with Ca and needle Ca and K concentrations, and negatively with soil NO_3 and needle N, S and Zn concentrations. Generally seed viability, needle Ca and K concentrations were low. Soil Al/P ratio was a better predictor for needle P than soil P concentration indicating that Al inhibits P uptake.

Conclusion We conclude that 1) N eutrophication reduces seed viability, but increases growth, 2) nutrient leaching reduces nutrient uptake, seed viability and subsequently growth, and 3) Al mobilisation reduces P uptake and indirectly growth. N deposition amplifies these mechanisms and therefore inhibits

Responsible Editor: Wen-Hao Zhang.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06857-7.

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regeneration and survival of juniper populations in western Europe.

Keywords Acidification · Aluminium · *Juniperus communis* · Foliar nutrient concentrations · Nitrogen · Regeneration

Introduction

The common juniper, *Juniperus communis*, is the most widely distributed conifer in the world (Enescu et al. 2016). However, in western Europe *J. communis* populations are rapidly declining (Clifton et al. 1997; Hüppe 1995; Sparrius et al. 2014; Verheyen et al. 2005). Several causes of decline are identified including habitat destruction, shading by woodland encroachment, fire, and pathogens such as *Phytophthora austroceadrae* (Green et al. 2015; Thomas et al. 2007). The populations contain a high genetic diversity, it is therefore unlikely that the decline is caused by inbreeding depression (Oostermeijer and De Knegt 2004; Reim et al. 2016; Vanden-Broeck et al. 2011). Since populations consist of old, even-aged trees, the major threat for European populations is the lack of natural regeneration (Clifton et al. 1997; European Commission 2009; Ward 1982).

Two important bottlenecks that seem to limit regeneration are 1) a low seed viability (Gruwez et al. 2013; Verheyen et al. 2009) and 2) a lack of suitable microsites for seedling development (Broome et al. 2017; De Frenne et al. 2020; Thomas et al. 2007). Seed viability is extremely low in Western-Europe ($<1\%$) (Gruwez et al. 2014) and is a limiting factor in juniper recruitment (De Frenne et al. 2020; Gruwez et al. 2013; Verheyen et al. 2009). Seed viability has been shown to be negatively associated with N deposition, climate warming and predation (Gruwez et al. 2014; Verheyen et al. 2009). The viable seeds need a suitable microsite to germinate and survive. Several studies show that microsites seem to be less suitable under water and light limitation, competition with fast growing plants, high soil N concentration and low soil cation saturation and pH (Broome et al. 2017). Except for water limitation, all these factors are directly or indirectly influenced by N deposition.

N deposition causes soil eutrophication and acidification, which leads to nutrient leaching and mobilization of toxic elements. Soil eutrophication with N stimulates N-limiting plants species, e.g. tall-grasses, which then outcompete slow growing species as *J. communis* (Kooijman et al. 2017; Stevens 2016). During soil acidification the acid components displace mono- and divalent cations (e.g. K^+, Na^+, Mg^{2+}, Ca^{2+}) from the soil adsorption complex. These important nutrients can leach to deeper soil layers where they become unavailable for plants. As soil acidification continues, Al dissolves in the soil solution, which can be harmful for plants (Rice and Herman 2012): Al inhibits growth (especially of roots) and can reduce Ca, K, Mg and P uptake (Gessa et al. 2005; Kochian et al. 2005; Mariano and Keltjens 2005; Singh et al. 2017). At the same time Ca, Mg and P can alleviate Al toxicity (Bose et al. 2011; Iqbal 2014; Liu and Xu 2015). As Al and macronutrient cations interact, for example at the plasma membrane surface, the macronutrient cation/Al ratio in the soil is a widely used ecological indicator for Al stress and nutrient imbalance (Brunner and Sperisen 2013; Cronan and Grigal 1995; Rahman et al. 2018), also for trees in acid soils (Vanguelova et al. 2007). Besides, needle nutrient concentrations are commonly used as indicator of tree health, since they represent the current nutrient status of trees (Cape et al. 1990; Talkner et al. 2019).

N deposition (via soil eutrophication and soil acidification) might thus affect the regeneration of *J. communis* by altering suitable microsites for saplings and reducing seed viability. The impact of N deposition on the performance of young saplings is, however, hardly studied, partly because poor germination rates in both natural areas and experiments impede statistical analysis (Broome et al. 2017). Gruwez et al. (2017) showed that seed viability and needle N, P and Ca concentrations were negatively correlated with acidifying deposition across populations in western Europe. Low seed viability is seen as one of the key factors limiting the number of *J. communis* seedlings (Verheyen et al. 2009). The responsible mechanisms behind these relations between N deposition on the one hand and plant performance and plant elemental composition on the other, however, remain unclear. The most likely mechanisms are: increased competition due to eutrophication, reduced availability of nutrients due to leaching, and increased toxicity via mobilisation. Combined monitoring of nutrients and toxic elements in soil and plant tissue and plant performance would give insight in which mechanism(s) is/are the most important.

In this study we investigated how soil nutrient concentrations, needle nutrient concentration, growth and seed viability in *J. communis* are associated with each other. We collected soil and needle samples in 72 female adult and 80 juvenile *J. communis* plants across six natural areas in the Netherlands and Belgium. We hypothesised that: 1) soil nutrient concentrations are associated with needle [E], growth and seed viability, 2) soil and needle aluminium concentrations are negatively associated with growth and seed viability and 3) soil Al/nutrient ratios are negatively associated with needle [E], growth and seed viability.

Methods

Study sites

Six natural areas with large *Juniperus communis* populations in the provinces of Drenthe (the Netherlands) and Limburg (Belgium) were investigated, namely Drouwenerzand, Dwingelderveld, Kampsheide and Mantingerzand in Drenthe (NL), and Heiderbos and De Teut in Limburg (BE) (see SI Table 1 for coordinates). All sites were on comparable soils that consist of dry, acidic, nutrient-poor, niveo-eolian sand deposited during the late Pleistocene (see SI Table 1) with typical heathland vegetation dominated by *Caluna vulgaris*, *Empetrum nigrum* and *Molinia caerulea*. N deposition in these areas range from 15 to 25 kg N/ha in 2018 / 2019 (RIVM; VMM).

Sampling of 152 Junipers

In each area, at least 12 juvenile and 12 female adult trees were sampled in the autumn between 2017 and 2019 (except for Kampsheide, where all available 4 juveniles were sampled). During a one day survey juveniles, i.e. individuals of 10–100 cm height, were located whereof twelve were randomly selected for sampling. Juveniles < 10 cm were not considered as these are easily overlooked in the field, and are too vulnerable for needle sampling. For the adults we decided sample only females, on the one hand as males and females differ in their growth and reproduction (Ward 2010) and their nutritional status (Rabska et al. 2020) and on the other as seeds ripen for two to three year on the females. To select female adults, twelve new coordinates were randomly selected and the closest adult female—individuals > 1.2 m with detectable female cones—to each coordinate was used in this study. Of each tree, we recorded basic information such as height (cm) and twig last-year growth in length using the bud scale scar (cm). For juveniles we determined the average growth of four twigs, whereas for adults we used the average growth of four twigs per cardinal direction (4 twigs × 4 cardinal directions = 16 measurements per adult). In addition, we sampled needles, seed, and soil as described below.

Needle samples

To determine element concentrations of the needles, 20 one-year-old twigs were collected. For smaller or fragile individuals we collected 100 one-year-old needles instead to minimize the impact of destructive sampling for the tree. The plant material was stored at 4 °C for maximum 2 days until fresh weight was determined. Twigs and needles were dried at 60 °C for at least 48 h to determine dry weight. Needles were taken from the twigs and grinded using a Foss Cyclotec™ 1093 sample mill or a Retch MM2 Pulverizer mixer mill (50 Hz, 3 min) to avoid loss of material. 5 mg grinded needle material was used for C/N analysis using the same procedure as for the soil samples. 50–200 mg grinded needle material was digested for 17 min with 5 ml sulphuric acid (HNO_3 65%) and 2 ml hydrogen peroxide (H_2O_2 30%) using a Milestone microwave (type ms 1200 mega). Digestates were diluted to 25, 50 or 100 ml with demineralized water and stored at 4 °C. Solutions were analyzed using an Inductively Coupled Plasma Spectrophotometer (ICP-OES, ICAP 6300, Thermo Fisher Scientific) to determine concentrations of Al, Ca, Fe, K, Mg, Mn, P, S, Si and Zn.

Seed samples

From 53 (female) adults in 5 areas at least 20 blue cones per individual were collected. Most populations had similar number of seeds structures per cone (Dw 2.73 ± 0.19, He 2.77 ± 0.16, Ka 2.70 ± 0.36, Ma 2.85 ± 0.13). Only Teut had significantly less seeds per cone (1.58 ± 0.40). Seeds were cut in half to count the number of empty and filled seeds (SI Fig. 1). Seeds that contain a white embryo and mega-gametophyte were classified as filled (similar to Verheyen et al. (2009)). Seed that contains brown shrivelled content were classified as empty. Per tree the total number of filled seeds was divided by the total number of seeds to calculate the filled seed (%). The percentage of filled seeds is generally used as a measure of seed viability (Verheyen et al. 2009).

Soil samples

To determine the soil [E], one soil sample (top 25 cm) was taken within 1 m from each *J. communis* tree ($n = 152$). Soil samples were stored at 4 °C. To determine the moisture content and soil organic matter a subsample was weighted and dried for 48 h at 60 °C and 4 h at 480 °C. Soil was grinded using a IKA Micro Pulverizer MFC 5000 rpm. To determine the C and N concentrations, 40 mg of dried grinded soil was rolled into a ball of tin foil and placed in an CNS element analyser (EA100 van Carlo Erba-Thermo Fisher Scientific). To determine the plant available element concentration a NaCl extraction was performed. A subsample of 17.5 g was shaken with 50 ml 0.2 *M* NaCl for 2 h and subsequently the solution was filtered with Rhizons SMS (Eijkelkamp Agrisearch Equipment, the Netherlands). The pH_{NaCl} of the solution was measured (E 512, Metrohm). Fifteen ml of the filtered solution was stored at -20 °C until NO_3^-, NH_4^+ and PO_4^{3-} concentrations were determined using a Seal auto-analyser III, Cl^- using a Bran + Luebbe auto-analyser III and Na^+ an K^+ using a Sherwood model 420 Flame Photometer. Ten ml of the filtered solution was acidified with 0.1 ml nitric acid (HNO_3 65%) and stored at 4 °C until the solution was analysed using an Inductively Coupled Plasma Spectrophotometer (ICP-OES, ICAP 6300, Thermo Fisher Scientific) to determine salt extractable concentrations of Al, Ca, Fe, K, Mg, Mn, P, S, Si and Zn.

Data analysis

All data analysis was performed in R version 4.0.0 (R Development Core Team 2020). Means and standard deviations of soil [E] (mmol/kg DW) and needle [E] (g/kg DW) were calculated per area for each element for both juveniles (SI Table 2a and 3a) and adults (SI Table 2b and 3b). One way-Anova and Tukey HSD tests were performed to test whether element concentrations differed between the study areas, for juveniles and adults separately. Element concentrations were log transformed to meet test assumptions.

We performed linear mixed effects models with area as random factor to test the effect of each soil [E] on each corresponding needle [E], growth and percentage of filled seeds, using the 'lme4' and 'lmerTest' package (Bates et al. 2015; Kuznetsova et al. 2017). The effects of needle [E] on growth and seed viability were tested similarly. To test the effect of soil Al_{NaCl} on the uptake of Ca, K, Mg and P, similar models were used with soil Al_{NaCl}/Ca_{NaCl}, Al_{NaCl}/K_{NaCl}, Al_{NaCl}/Mg_{NaCl} and Al_{NaCl}/P_{NaCl}-ratios as fixed factor and needle Ca, K, Mg and P as dependent variable, respectively. Seed viability was log transformed to meet assumptions for normality and homogeneity.

For juveniles, we used relative height growth rate (RGRh, cm/cm/yr) as – in contrast to adults – the growth of juveniles was associated by how tall they were. As junipers reach maturity they start investing in the production of cones at the cost of their vegetative growth rate (Iszkuło and Boratyński 2011). Therefore for adults absolute growth (cm/yr) was used instead of RGRh. The limiting chi square distributions of the likelihood ratio test were used to calculate p-values to determine which association were significant ($p < 0.05$) and marginally significant ($0.05 < p < 0.10$) (Kuznetsova et al. 2017).

Results

Study areas varied significantly in soil [E] (Fig. 1, SI Table 2a,b) and needle [E] (Fig. 2, SI Table 3a,b). Average growth was 4.8 ± 2.0 cm and 3.8 ± 1.5 cm for juveniles and adults, respectively. Percentages of filled seeds of adults was surprisingly high in Teut 52.9 ± 34.4%, but low in the other areas 6.5 ± 12.8% (Fig. 3).

Soil [E] were associated with juvenile needle [E] which in turn were associated with shoot height growth. Most soil [E] (Ca_{NaCl}, Mn_{NaCl}, P_{NaCl}, S_{NaCl}, Zn_{NaCl} and inorganic N_{NaCl}) were reflected by corresponding needle [E] in juveniles, whereas soil Al_{NaCl}, Al_{NaCl}/Ca_{NaCl} and Al_{NaCl}/P_{NaCl} were negatively associated with Al, Ca and P in needles, respectively (Table 1 and SI Table 4a). Subsequently, most of these needle [E] (K, N, P, S, Zn (all significant) and

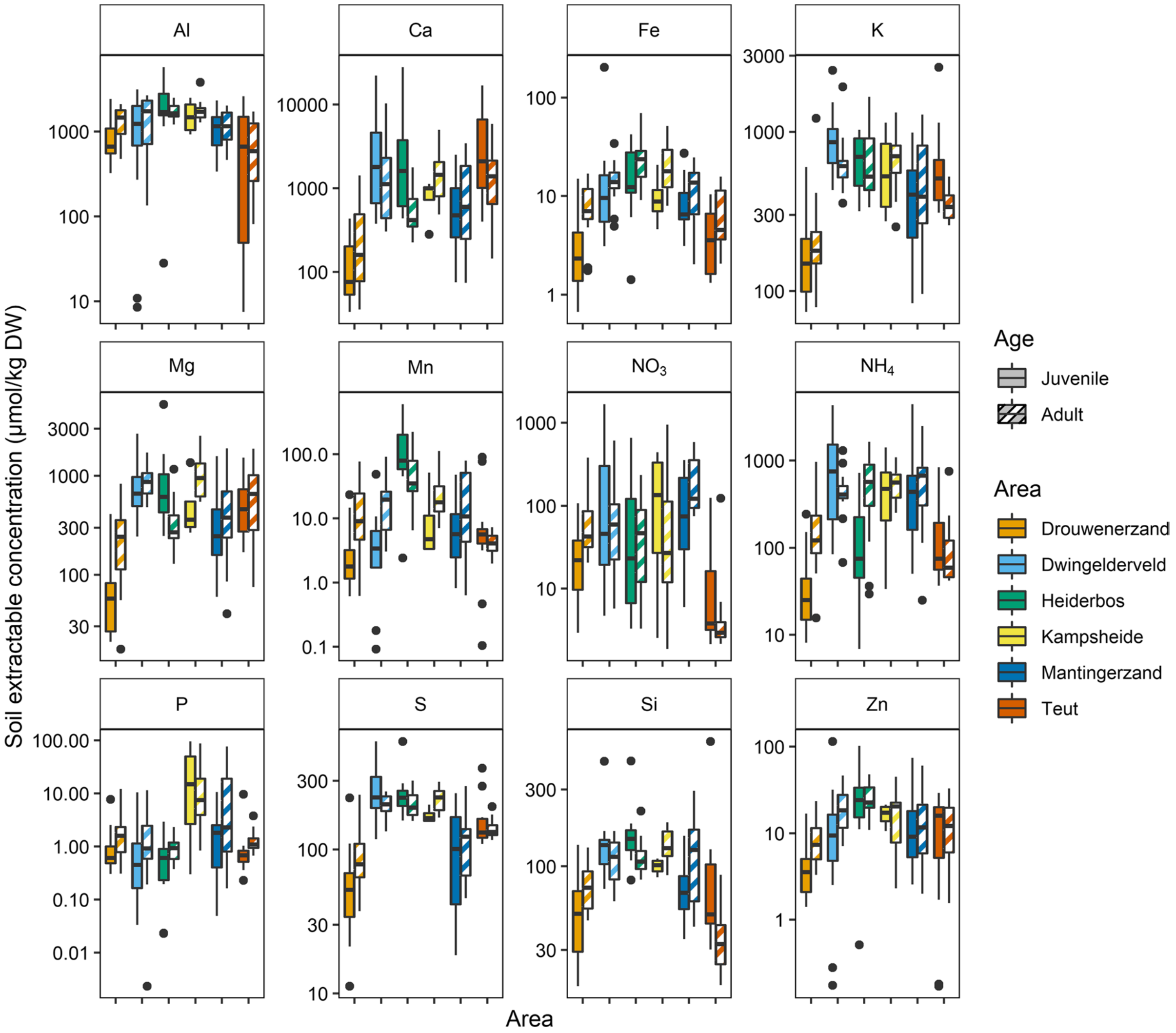

Fig. 1 Soil element concentration (µmol/kg DW) of aluminium (Al), calcium (Ca), chloride (Cl), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), nitrate (NO_3), ammonium (NH_4), phosphorous (P), sulphur (S), silicon (Si) and Zinc (Zn) (g/kg DW) per area (in colour). Juveniles and adults are represented by filled and striped patterns, respectively. For statistical differences between areas per element for juveniles and adults see SI Table 2a and 2b, respectively. Lower and upper box boundaries represent 25th and 75th percentiles, respectively, line inside box represents the median, lower and upper error lines represent 10th and 90th percentiles, respectively, filled circles data falling outside 10th and 90th percentiles

Ca (marginally significant)) were positively associated with RGRh, whereas Si was negatively associated with RGRh. Direct associations between soil [E] and RGRh were scarce; soil N/P ratio was positively and Zn_{NaCl} was marginally negatively related to RGRh.

In adults, similar patterns were found (Table 1 and SI Table 4b). Several soil [E] (namely Ca_{NaCl}, K_{NaCl}, Mn_{NaCl}, $N_{inorgNaCl}$, P_{NaCl}) were positively associated with corresponding needle [E] (all significant, except for P (marginally significant)) and, mainly for other elements, needle [E] and growth were positively associated (Mg, P, S, Zn, N (all significant) and K (marginally significant)). Seed viability (% of seeds that were filled) was positively associated with soil Mg_{NaCl}, soil Ca_{NaCl}, needle Ca and needle K, and negatively with soil $NO_3^-{}_{NaCl}$ and needle N, S, Zn, and Si, where the associations with Ca, K and Si were marginally significant. Direct associations between soil [E] and height growth or seed viability were absent. Soil Al/Ca and Al/P ratios

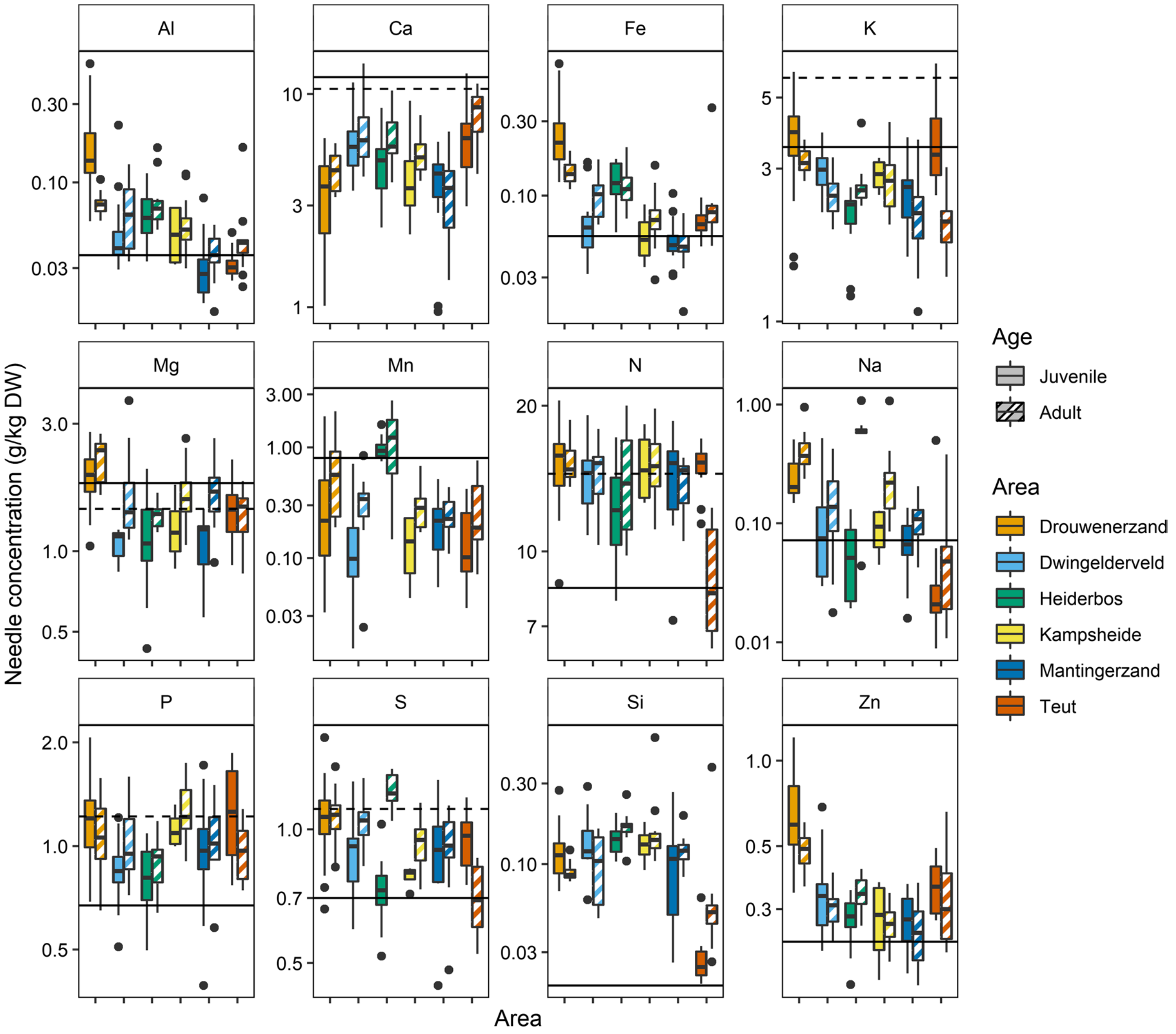

Fig. 2 Needle concentration of aluminium (Al), calcium (Ca), chloride (Cl), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorous (P), sulphur (S), silicon (Si) and Zinc (Zn) (g/kg DW) per area (in colour). Juveniles and adults are represented by filled and striped patterns, respectively. To be able to compare with literature, solid and dashed horizontal lines represent average element concentrations in adults reported by Penuelas et al. (2001) and Gruwez et al. (2017), respectively. For statistical differences between areas per element for juveniles and adults see SI Table 3a and 3b, respectively. Lower and upper box boundaries represent 25th and 75th percentiles, respectively, line inside box represents the median, lower and upper error lines represent 10th and 90th percentiles, respectively, filled circles data falling outside 10th and 90th percentiles

were negatively correlated to needle Ca and P concentrations, respectively. Soil Al/Ca and Al/Mg were marginally positively associated with height growth. Seed viability was negatively associated with soil Al/Mg and marginally negatively associated with soil Al/Ca. In general, needle [E] and seed viability were more strongly associated with the corresponding soil [E] than the corresponding Al/[E] ratio, except for needle P, which was better associated with soil Al/P ratio than the soil P concentration in both adults and juveniles.

Discussion

Previous studies showed that nitrogen deposition is associated with a lack of recruitment of *J.*

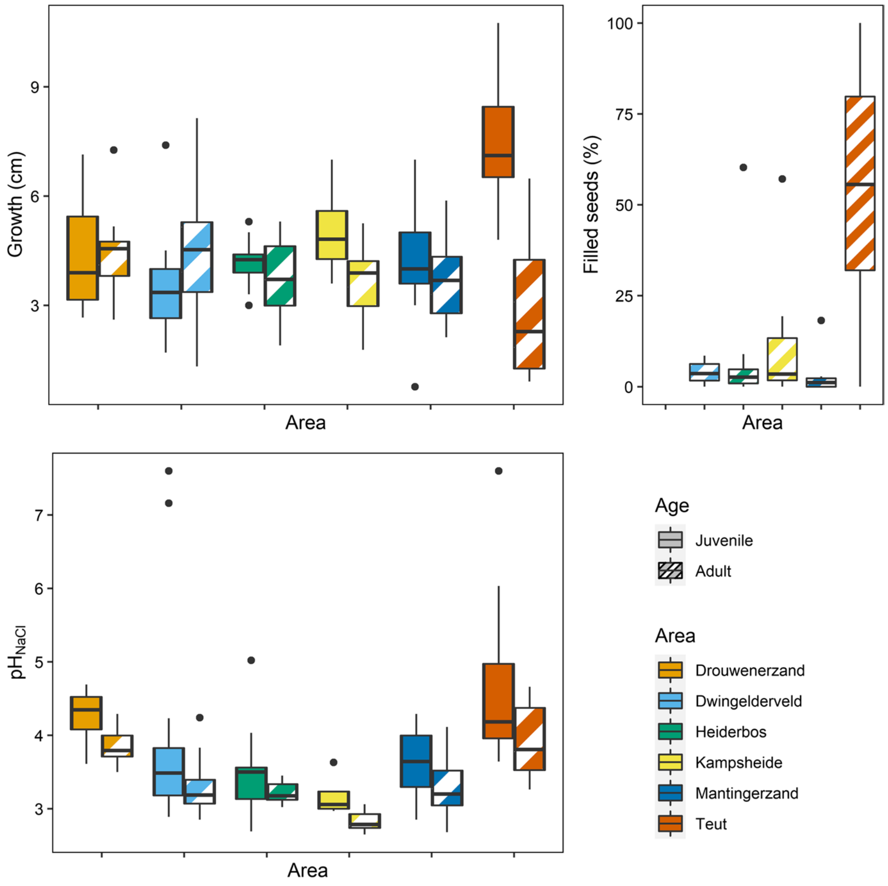

Fig. 3 For adults (striped) and juveniles (filled) per area are shown: Growth (cm/yr), Filled seeds (%) and soil pH_{NaCl}. As juveniles did not (yet) produce any seed data for filled seed (%) was not determined. Colours represent different areas. Lower and upper box boundaries represent 25th and 75th percentiles, respectively, line inside box represents the median, lower and upper error lines represent 10th and 90th percentiles, respectively, filled circles data falling outside 10th and 90th percentiles

communis, which is threatening the existence of populations in Western Europe. To better understand the mechanisms underlying N deposition impacts the recruitment of *J. communis*, we investigated the associations between soil [E], needle [E], growth and seed viability in juveniles and adults of *J. communis* in the field. Generally we found that soil [E] positively affected needle [E], in both adults and juveniles, which in turn were positively associated with their growth. In female adults, the percentage of filled seeds was positively associated with soil Mg concentration, marginally positively with soil Ca and needle Ca and K concentrations, and negatively with soil NO_3 and needle N, S and Zn concentrations. Based on our findings, we discuss how N deposition, via N eutrophication, cation leaching and metal mobilisation, affects *J. communis* seed viability and sapling performance.

N eutrophication

For both adults and juveniles, soil NH_4 and NO_3 were associated with needle N which in turn was associated with growth. As in many plants and ecosystems, N seems to stimulate the growth of *J. communis*. However, an increase in soil N, by N deposition, does not necessarily stimulate regeneration of *J. communis* as it can increase competition with fast growing tall grasses as *Molinia caerulea* (Kooijman et al. 2017). Average growth was 4.8 ± 2.0 cm and 3.8 ± 1.5 cm for juveniles and adults, respectively, which is comparable to previous findings on growth of *J. communis* in North-Western Europe (Broome 2003; Ward 2007; Ward and Shellswell 2017).

Seed viability was negatively associated with NO_3 and N concentrations in soil and needles, respectively. Verheyen et al. (2009) showed that percentages

Table 1 Summary of mixed effect models

	Linear mixed model	Mg	K	Ca	Mn	N	NO_3	NH_4	P	S	Zn	Al	Si	Fe	Al/Ca	Al/P	Al/K	Al/Mg	N/P
juveniles	needle [E] ~ soil [E] + (1 + area)			+	+		+	+	+	+	+	-			-	-			
	RGRh ~ needle [E] + (1 + area)		+	(+)		+			+	+	+		-						
	RGRh ~ Soil [E] + (1 + area)										(-)								+
Adults	needle [E] ~ soil [E] + (1 + area)		+	+	+		+	+	(+)						-	-			
	growth ~ needle [E] + (1 + area)	+				+			+	+	+								+
	growth ~ Soil [E] + (1 + area)														(+)			(+)	
	Filled seed (%) ~ needle [E] + (1 + area)		(+)	(+)		-				-	-		(-)						(-)
	Filled seed (%) ~ soil [E] + (1 + area)	+		(+)			-								(-)			-	

Each row shows a different model with datasets of juveniles and adults. Columns represent different elements that were tested. For Al/Ca, Al/K, Al/Mg and Al/P we tested associations of these element ratios in the soil and the Ca, K, Mg and P concentrations in the needles, respectively. Significant effects of the fixed factor to the dependent variable are indicated by + and -, marginally significant effects ($0.05 < p$ (Chi) < 0.1) are shown between brackets. See SI Table 4 for df, t-values and p-values for each test. Element in grey cells were not investigated

of filled seeds are low in the Netherlands, Belgium and Northern France compared to the rest of Europe (e.g. Germany, Spain and Sweden). The percentage of filled seeds was low in our study areas as well (6.5 ± 12.8%), except for the Teut (52.9 ± 34.4%). Needle N concentrations in Teut adults were significantly lower than in the other study areas. These findings are in line with Gruwez et al. (2017) who studied associations between needle [E] and seed viability in twenty *J. communis* populations across Europe and found a negative association between needle N and seed viability. Based on the found associations we conclude that N eutrophication seems to reduce seed viability and increase height growth rate, although N might stimulate growth of competitors even stronger.

Nutrient leaching

We found that many soil [E] were associated with needle [E] for both juveniles (Ca, Mn, Ninorg, P, S, Zn) and adults (Ca, K, Mn, Ninorg), indicating that nutrient uptake depends on nutrient availability in the soil. Needle [E] in turn were positively associated with plant performance in terms of growth in both juveniles (Ca, K, N, P, S, Zn) and adults (Mg, N, P, S, Zn). Therefore it seems that further leaching of nutrients, (enhanced by N deposition) likely hampers nutrient uptake and growth. Braun et al. (2020) found similar patterns in *Fagus sylvatica* (European beech) in which foliar K, P, Mg and Mn are associated with soil K, P, Mg and Mn, respectively. Thereby they found that foliar concentrations of K, P, N and Mg declined with long term N deposition (Braun et al. 2020).

Unlike juveniles, needle [E] of P, S, Zn in adults were not correlated with soil [E] and effect sizes were smaller for most elements (t-values were closer to zero). Adults can store nutrients in their stem and roots, therefore needle [E] could be less related to actual nutrient uptake in adults (Aoyagi and Kitayama 2016). Also, because of that storage in stems and roots, adults might be better able to regulate uptake or exclusion of certain elements.

We found that seed viability was positively associated with macronutrient cations in needles (Ca, K) and soil (Ca, Mg), and negatively with needle concentrations of N, S, (Si) and Zn. This is largely in agreement with Gruwez et al. (2017), who found that seed viability of *J. communis* was positively associated with needle Ca, Mg and P concentrations and negatively with needle N and S. In contrast to our results, Gruwez et al. (2017) found a negative association with needle K. Unlike Gruwez et al. (2017), the needle K concentration was extremely low in our populations. A K deficiency would explain the low seed viability of our populations. Note that in contrast to Gruwez et al. (2017) who investigated differences among European populations, we investigated differences within populations.

Most of our needle [E] were comparable with values found in adults across Europe (Gruwez et al. 2017; Penuelas et al. 2001). However, our Ca, K and Mn concentrations were lower and Al, Fe, Si and Zn concentrations were higher than found by Gruwez et al. (2017) and Penuelas et al. (2001). Especially needle Ca and K concentrations were low in our study areas (Ca, 5.80 ± 2.45; K, 2.55 ± 0.63 g/kg DW in adults) compared to European averages in adults (Ca, 10.59; K, 5.76 g/kg DW (Gruwez et al. 2017)). Deficiency thresholds for juniper element concentrations are not available. But when we compare our element concentrations to nutrition deficiency thresholds of other coniferous species as Norway spruce and Scots pine, especially K concentrations were low (Talkner et al. 2019). The lower nutrition threshold for Ca was 2.0 g/kg DW for Norway spruce and Scots pine and 5.0 g/kg DW for European beech and Oak (Talkner et al. 2019). Our K concentrations were lower than the critical deficiency thresholds of 3.5 g/kg DW in both Scots pine and Norway Spruce (Talkner et al. 2019), thus unless the threshold for common juniper is lower, our junipers suffered from K deficiency. Thereby the juvenile needle K concentrations were strongly associated with RGRh. Lucassen et al. (2011) found that, among others, the K concentration in adult needles was related to the number of seedlings in Dutch populations. These findings support the idea that K limits juniper performance and regeneration in this region.

Concluding, junipers in the investigated areas have low Ca and K concentrations and seem to suffer from K deficiency. Thereby seed viability of adults and relative height growth rates of juveniles were associated with needle and/or soil concentrations of Ca, K, and Mg. Leaching of these exchangeable cations, stimulated by N deposition, is likely to hamper juniper rejuvenation by reducing seed viability as well as sapling performance.

Al mobilisation

Soil and needle Al concentrations were not associated with juniper growth or seed viability. Against our hypothesis needle Al concentrations in juveniles were even negatively associated with soil Al concentrations. Soil Al can suppress root elongation and thereby could also limit the uptake of other elements (Rahman et al. 2018; Singh et al. 2017). Soil Al/P ratio was negatively associated with needle P concentration, in both juveniles as in adults. It is likely that Al reduces P uptake, as needle P concentration was more strongly associated with soil Al/P ratio than soil P itself. Al can immobilize P at the root surface which could lead to a reduced uptake (Gessa et al. 2005). Al can also reduce P uptake by harming mutualistic interactions with mycorrhizal fungi which can support the P uptake (Lenoir et al. 2016).

We did not find associations between soil Al or Al/Ca, Al/Mg, Al/K ratios and juniper performance in terms of needle [E], seed viability and height growth, and if so, the associations between the performance and the exchangeable cation concentration itself were stronger. For instance, the soil Al/Mg ratio (significantly) and Al/Ca (marginally significant, $p < 0.1$) were negatively associated with seed viability. However, soil Ca and Mg concentrations were stronger associated with seed viability than soil Al/Ca and Al/Mg ratios. As 1) soil Ca is associated with needle Ca and 2) soil Al/Ca ratio depends on soil Ca, it is likely that Al/Ca ratio is associated with needle Ca even if soil Al would not affect Ca uptake at all. Thus even though Al/Mg and Al/Ca ratios are negatively associated with seed viability the role of Al remains unclear. Concluding, in our study areas there are indications that soil Al reduces P uptake, whereas uptake of other nutrients and height growth seemed not to be affected by soil Al.

We found associations between soil [E] and needle [E] as well as needle [E] and height growth, while direct associations between soil [E] and height growth were absent. This can be due to imperfect correlations between soil [E] and needle [E] on the one hand and needle [E] and height growth on the other. Imperfect correlations between soil [E] and needle [E] can be caused by (i) nutrient uptake regulation by plants (Marschner and Marschner 2012), (ii) interference of other elements with nutrient uptake (e.g. Al) (Gessa et al. 2005;

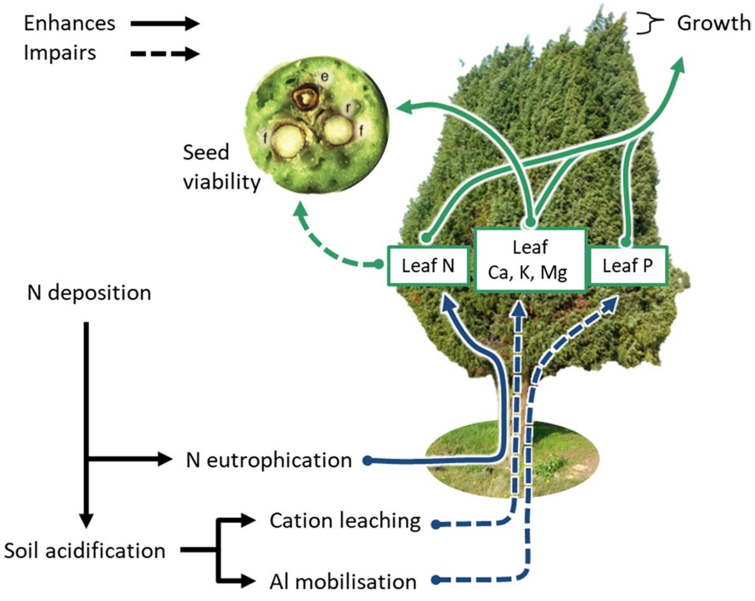

Fig. 4 Overview of the associations between consequences of N deposition and *J. communis* performance. Solid and dotted lines represent positive and negative associations respectively

Kochian et al. 2005), (iii) presence of soil microorganisms that affect nutrient uptake (e.g. mycorrhizal fungi) (Latef et al. 2016; Veldhuis et al. 2022) and (iv) allocation of nutrients to other tissues (e.g. stem or roots) instead of the needles (Aoyagi and Kitayama 2016). Similarly height growth and seed viability do not solely depend on needle [E] (and/or soil [E]) but are affected by many other factors, which are not necessarily related to N deposition. Height growth is also affected by e.g. temperature, drought, irradiation, reproductive effort element interactions, pathogens and pests (Iszkuło and Boratyński 2011; Suzuki et al. 2014). Seed viability can – besides consequences of N deposition – be affected by age (Ward 2010), infection by insects (Gruwez et al. 2014), temperature (Gruwez et al. 2014, 2016; Verheyen et al. 2009; Ward 2010) and fertilization issues (Kärkkäinen et al. 1999). Quality and quantity of pollen (Pers-Kamczyc et al. 2020, 2022) as well as the metabolites in pollen (Pers-Kamczyc and Kamczyc 2022) in *J. communis* are affected by soil fertilization. This indicates that N deposition can affect male pollen, subsequently fertilization and later on seed viability of the females. Whether pollen quality strongly reduces seed viability in natural populations needs further investigation. As our population consist of many individuals and the male/female ratio was approximately 50/50, we assumed the females received a mix of pollen of many males. Altogether, besides element concentrations, several other factors could have influenced our plant performance parameters.

Summarizing, we found that consequences of N deposition – N eutrophication, nutrient leaching and Al mobilisation – are negatively associated with plant nutrient status, height growth and/or seed viability of *J. communis* (Fig. 4). N eutrophication, seems to increase height growth but reduces seed production. Nutrient leaching likely reduces nutrient uptake and subsequently lowers juniper performance, both in terms of height growth and production of viable seeds. In our study areas, we found low macronutrient cations (Ca, K, Mg) in needles, low seed viability and a positive association between these two. Therefore, it is likely that these nutrients currently limit the performance of *J. communis* in terms of seed production and thereby limit regeneration. Leaching of these nutrients, amplified by ongoing N deposition and subsequent soil acidification, likely reduces height growth and regeneration of *J. communis*. Mobilization of Al seems to reduce P uptake, which subsequently may result in height growth limitation, whereas the uptake of exchangeable cations, seed viability and height growth were not affected by soil Al. Via these three described mechanisms, N deposition is a the major threat for *J. communis* populations on nutrient poor and poorly buffered heathland soils in North Western Europe, and could remain so if no measures are taken to reduce the N deposition load, as well as to improve soil quality (e.g. by sod cutting, liming, rock powder Holland et al. 2018; Ramos et al. 2021)). However, restoration measures that replenish the soil cation availability should be tested to verify the causal relationships among soil[E] and juniper performance, and to investigate their effectiveness as restoration tool for juniper recruitment.

Acknowledgements We thank Conservation ecology group of GELIFES and BWARE for using their lab facilities, N. Eck and Frank Luijckx for their help in the lab and field respectively. We thank Het Drentse Landschap, Natuurmonumenten, Agentschap Natuur en Bos, for their permission to collect samples in the natural areas. Financial support was provided by Province of Drenthe and Agentschap Natuur en Bos. The authors have no known conflicts of interest to declare.

Authors' contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Rik Veldhuis. The first draft of the manuscript was written by Rik Veldhuis and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

The authors have no relevant financial or non-financial interests to disclose.

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