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ORIGINAL ARTICLE

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Climate warming and atmospheric deposition affect seed viability of common juniper (*Juniperus communis*) via their impact on the nutrient status of the plant

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Abstract Global environmental change is increasingly affecting species worldwide. One of the emblematic casualties among plants in several European countries is common juniper (*Juniperus communis*). Many populations of common juniper throughout its distribution range are declining. The relative lack of viable seed production, resulting in low probabilities for successful natural regeneration, is one of the main reasons for this decline. Climate warming and elevated atmospheric depositions have been shown to negatively affect seed viability of common juniper, but our understanding of the underlying mechanisms remains scarce. One possible pathway is via changes in the plant nutrient status that, in turn, may affect seed viability. Here we took advantage of large-scale gradients in climate and atmospheric depositions between central Sweden and northern Spain, and analysed foliar nutrient concentrations and stoichiometry and seed viability in 20 juniper populations spread across Europe. Our results show that increasing temperatures can negatively affect needle N and P concentrations while enhanced potentially acidifying depositions resulted in lower foliar N and Ca concentrations. Needle C:N ratios increased with higher temperature, acidifying depositions and precipitation. By linking these patterns to seed viability, we found that low needle P, Ca and Mg concentrations were related to low seed viability. Thus, a shortage of these key elements during seed development and seed nutrient storage, can lead to anomalies and seed abortion. These findings help to explain the low seed viability of juniper in Europe and may help to assist land managers to take urgently needed conservation actions.

Keywords *Juniperus communis* · Seed viability · Global change ecology · Stoichiometry · Climate change

Introduction

An ever increasing mixture of anthropogenic stressors is acting on ecosystems worldwide. Two of the most important threats to biodiversity and ecosystem functioning are elevated nitrogen (N) inputs and climate warming. Worldwide atmospheric deposition of biologically reactive N more than tripled from 1860 to the early 1990s and by the year 2050 a 2.4 to 2.7-fold increase in eutrophication of terrestrial ecosystems is predicted, probably causing an unprecedented loss of biodiversity and subsequent biotic homogenisation of ecosystems (Tilman et al. 2001). This decades-long N deposition is partly responsible for the already significant losses of terrestrial plant diversity (Vitousek et al. 1997; Clark and Tilman 2008; De Schrijver et al. 2011). Models also forecast strong climate-change impacts on biodiversity during the 21st century (Warren et al. 2013).

Increasingly, the common juniper (*Juniperus communis*), a coniferous tree or shrub with one of the widest distribution ranges of all plant species (Adams 2008), emerges as one of the emblematic casualties of global change (García 2001; Verheyen et al. 2009; Gruwez et al. 2014, 2016). Many populations throughout its distribution range are declining in size and number, including those in Belgium, the Netherlands, northern and western

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Germany, England and Mediterranean mountain regions (e.g. Clifton et al. 1997; García et al. 1999; Oostermeijer and De Knegt 2004). Conversely, the species is still abundant and exhibits good regeneration in parts of the Alps, Scandinavia and Poland (Falinski 1980; Rosén 1995; Rosén and Bakker 2005). Nevertheless, due to the threatened status in several regions in Europe *J. communis* communities are listed in Annex I of the EU Habitat Directive (code 5130).

The relative lack of viable seed production, resulting in low probabilities for successful natural regeneration, is one of the main reasons for this decline (Verheyen et al. 2005, 2009; Gruwez et al. 2014). Two global-change drivers, i.e. climate warming and enhanced airborne depositions of potentially acidifying substances such as N and sulphur (S), negatively affect seed viability of common juniper (Verheyen et al. 2009; Gruwez et al. 2014, 2016). In addition, altering precipitation patterns as a result of climate change can also influence seed viability. As the impact of drivers of global change are expected to increase in the coming decades (Rands et al. 2010), it is urgently needed to improve our understanding on the future responses of juniper to global changes in order to soundly inform policy and management decisions. Several studies have investigated the influence of different global-change drivers on sexual reproduction of plants, including warming (Peñuelas et al. 2004; De Frenne et al. 2011; Koivuranta et al. 2012), elevated CO_2 concentrations (Thurig et al. 2003), nitrogen deposition (Callahan et al. 2008) and drought (Demirtas et al. 2010). Fewer studies, however, have investigated the interacting effects of interacting drivers on sexual reproduction (Hovenden et al. 2008; HilleRisLambers et al. 2009; Verheyen et al. 2009; Li et al. 2011). Even less is known about the impacts on different processes acting during subsequent phases of the sexual reproductive cycle of plants (but see Owens et al. 2001; Hedhly 2011, Gruwez et al. 2014). For example, Gruwez et al. (2014) found that the negative effects of increasing temperatures act both after fertilization and during embryo development. Effects of potentially acidifying depositions were, however, only pronounced after embryo development. The mechanisms behind these effects and especially the nutrients status of the plant remain, however, unclear.

Several studies detected effects of temperature (Reich and Oleksyn 2004; Han et al. 2005; Zheng and Shangguan 2007; Yuan and Chen 2007; Kang et al. 2011) and N deposition (Innes 1995; Thimonier et al. 2010; Sardans et al. 2011; Blanes et al. 2013) on foliar concentrations of N and phosphorous (P). Far less is known, however, about the effects of these global-change drivers on tissue nutrient concentrations of potassium (K), magnesium (Mg), S and calcium (Ca) (but see Sardans et al. 2011). Foliar nutrient concentrations can be directly affected by temperature and N deposition, for instance, via different nutrient uptake, nutrient leaching from the leaves or indirectly via aluminium impacts at high soil acidity on root functioning and survival. Macronutrient concentrations in foliage, in turn, can influence seed production and quality. For example, foliage N, P and K concentrations showed positive correlations to the number of flowers in *Malus* spp. (Marschner 1995) and to the number of conelets and the seed weight in *Pinus sylvestris* (Karlsson and Örlander 2002). Indeed, the macronutrients N, P, S, K, Ca and Mg play an important role in plant growth and plant functioning (Marschner 1995). It is well known that N and S compounds accumulate as reserves during seed development (mostly as proteins), but also P, Mg, K and Ca are sequestered within mature seeds (Lott et al. 1995). In addition to serving as a reserve, K, Ca and Mg also fulfil regulatory roles (e.g. osmoregulation, cell extension and cell wall stabilisation) (Marschner 1995). For common juniper, Lucassen et al. (2011) found a relationship between the chemical composition of the needles and the seeds on the one hand, and the abundance of seedlings in Dutch populations (i.e. a positive relationship for K and P concentrations and negative for aluminium concentrations). Hence, a potential pathway by which temperature and atmospheric deposition impact seed viability is via their influence on soil and/or foliar nutrient concentrations. For instance, a potential mechanism is that a shortage in key elements during the nutrient storage stage of the seed and embryo development (e.g. P, Ca, Mg), can lead to anomalies and seed abortion. This could then potentially explain lower seed viability in certain parts of the distribution range. Here we specifically focus on the impact of the environment on the nutrient status of the plant and the impact of the latter on the seed viability. The innovative character of this study lies specifically in the relationship with foliar chemistry that advances our understanding of the actual pathways that inhibit seed viability.

To address this research gap, we performed a large-scale sampling campaign of needles and seeds of common juniper along wide temperature and N deposition gradients. We focused on two development phases [that is, seeds sampled (1) shortly after fertilization and (2) at the end of embryo ripening] in 20 common juniper populations spread across Europe (from Sweden to Spain and from the United Kingdom to Poland) (Fig. 1a). We took advantage of the wide climatic and atmospheric deposition gradients (De Frenne et al. 2013) in this area to study the influence of three global-change drivers (increasing temperature, potentially acidifying depositions and altering precipitation) on the macronutrient concentration in the needles. In addition, we link the macronutrient concentration in the needles to the viability of the seeds at two seed development phases. We specifically assessed the following hypotheses: (1) the three global-change drivers (warmer temperatures, enhanced atmospheric depositions and altered precipitation patterns) have a negative effect on the macronutrient concentrations in the common juniper needles and (2) these lower macronutrient concentrations are, in turn, related to declining seed viability of juniper in certain parts of its distribution range.

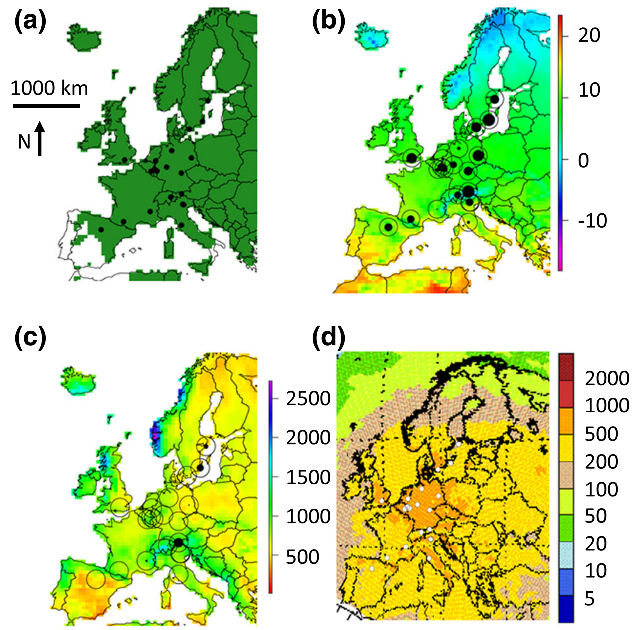

Fig. 1 **a** Location of the sampled common juniper populations (*circles*) and distribution range of the study species in Europe. **b** Mean annual temperature across the study area (°C) between 1950 and 2000 (WorldClim data, Hijmans et al. 2005). The partial filling of each circle denotes the proportion of viable seed phase 2 (SP2)-seeds in that respective population (i.e., *unfilled circle* 0% viable SP2-seeds, *completely filled circle* 100% SP2-viable seeds). **c** Mean annual precipitation across the study area (mm) between 1950 and 2000 (Hijmans et al. 2005). The partial filling of each circle denotes the proportion of viable seed phase 3 (SP3)-seeds in that respective population (i.e., *unfilled circle* 0% viable SP3-seeds, *completely filled circle* 100% viable SP3-seeds). **d** Mean atmospheric deposition of total oxidized nitrogen (mg year^{-1} m^{-2}) across the studied populations (*white circles*) between 2006 and 2010 using the EMEP database (http://www.emep.int). More information on the sampled populations is available in Table S1

Methods

Study species

Juniperus communis is a coniferous shrub or tree and one of the most widely spread plant species with a geographic distribution covering most of the northern hemisphere (Adams 2008). It is dioecious and wind-pollinated. The mature females annually produce fleshy, spherical, berry-like cones of approximately 6.5 mm in diameter whose maturation takes 2 or 3 years (García et al. 2000; Thomas et al. 2007; Ward 2010). The sexual reproduction starts with the cone initiation in autumn or early winter (Singh 1978) with the female strobili usually containing three ovules (Thomas et al. 2007). Common juniper has a dual seed ripening strategy, with both two- and a 3-year cycles occurring (Gruwez et al. 2013). In a 2 years-cycle, pollination takes place in the next spring and fertilization follows in the summer of the same year. After fertilization, the embryo development starts and the seeds are ready for dispersal by the end of the summer of the second year. In a three year cycle, fertilization is postponed by 1 year and only takes place in the summer of the second year, such that seeds are ready for dispersal by the end of the third summer (García et al. 2000; Thomas et al. 2007; Ward 2010; Gruwez et al. 2013). A detailed description including a schematic overview of the seed and cone development is available in Gruwez et al. (2013). Herein we refer to seeds from the developmental phase shortly after fertilization as seed phase 2 seeds (SP2 seeds), while seeds that have a ripe embryo are referred to as seed phase 3 seeds (SP3 seeds; Gruwez et al. 2013).

Sampling

Seeds of 20 populations across the species' distribution range in Europe (Fig. 1 and Table S1 in Electronic Supplementary Material) were sampled in autumn of 2010. Populations consisted of at least 30 individual shrubs growing in unshaded conditions (i.e., not below the canopy of other tree species). In each population three to five (average 4.8 ± 0.5 SD) cone bearing shrubs were randomly selected to exclude undesired co-variation of e.g. the shrub age and soil characteristics. Per shrub, three branches were randomly selected, of which on average 32.7 (±7.7 SD) SP2 seeds and 21.7 (±8.6 SD) SP3 seeds were sampled. In addition, from each branch, all the 1-year-old needles were collected and pooled per shrub (cfr. the manual of ICP-forest; Rautio et al. 2010). The 20 populations considered here consist of a subset of the populations used in Gruwez et al. (2014) for which needle nutrient concentrations were available.

Environmental variables

We compiled information on the average environment for each of the sampled juniper populations. Temperature was expressed as the number of growing degree days above 0 °C base temperature ($\mathrm{GDD}_{>0\,°\mathrm{C}}$; cf. Hall et al. 2002), which was calculated for the year preceding the sampling. Daily minimum and maximum temperatures of each population were obtained from the nearest weather stations (see Table S1) and used to calculate $\mathrm{GDD}_{>0\,°\mathrm{C}}$. When the population and the weather station had different altitudes, a mean adiabatic lapse rate correction of 5.5 K km^{-1} (Körner 2007) was applied. The $\mathrm{GDD}_{>0\,°\mathrm{C}}$ ranged between 1275.1 and 5074.7 with an average of 3333.9 (±861.9 SD).

Nitrogen and sulphur deposition data were obtained from the European Monitoring and Evaluation Programme database (EMEP) (http://www.emep.int). EMEP is the 'Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air pollutants in Europe' and provides scientific information on the emission, transport and deposition of air pollutants. Here, averaged data for 2006 to 2010 were used: total (wet + dry) inorganic nitrogen ($\mathrm{NH_x}$ + $\mathrm{NO_y}$)

depositions expressed as kg ha^{-1} year^{-1} and potentially acidifying (NH_x + NO_y + SO_x) depositions expressed as keq ha^{-1} year^{-1} in 50 × 50 km^2 grid cells covering Europe. Nitrogen depositions ranged from 4.85 to 28.13 kg ha^{-1} year^{-1} with an average of 13.19 kg ha^{-1} year^{-1} (±6.38 SD) and potentially acidifying depositions ranged from 0.51 to 2.38 keq ha^{-1} year^{-1} with an average of 1.25 keq ha^{-1} year^{-1} (±0.55 SD) (Fig. 1d).

Next, the average annual amount of precipitation in the 5 years preceding the time of sampling was calculated per population using the yearly precipitation data from EMEP. Average yearly precipitation ranged from 652.3 to 1693.4 mm year^{-1} with an average of 978.1 mm year^{-1} (±295.1 SD).

Finally, for each population we estimated two soil characteristics in different classes: texture of the topsoil (0–50 cm) (sandy, sandy loam, loamy, clayey) and bedrock type (calcareous vs. non-calcareous).

Needle analyses

Needles were dried to constant weight at 70 °C for 48 h. Concentrations of P, K, Mg and Ca were obtained after digesting 100 mg sample with 0.4 ml $HClO_4$ (65%) and 2 ml HNO_3 (70%) in Teflon pots or 4 h at 140 °C. Phosphorus was measured colorimetrically according to the malachite green procedure (Lajtha et al. 1999). K, Mg and Ca concentrations were measured by atomic absorption spectrophotometry (AA240FS, Fast Sequential AAS). The concentrations of N, S and C were measured using an elemental analyzer (Vario MACRO cube CNS, Elementar, Germany). The variables that were considered for further analyses were needle concentrations of N, S, C, P, K, Ca, Mg. Due to the importance of relative nutrient concentrations and stoichiometry, we also studied the ratios of C:N and N:P.

Seed analyses

The viability of all sampled seeds was assessed by means of stereoscopic observations of dissected seeds (3111 seeds for SP2 and 2063 seeds for SP3, in total; following Gruwez et al. 2013). Seeds that had no visible signs of anomalies were considered to have the potential to develop to the next phase and are further referred to as 'viable seeds'. Although this method tends to overestimate seed viability inferred from tetrazolium tests, results from both methods are strongly correlated (R = 0.681, n = 198, P < 0.01; Adriaenssens 2006). Viable SP2 seeds presented a megagametophyte and nucellus consisting of green-white and moist tissue, not completely filling the space within the seed coat (Gruwez et al. 2013). Viable SP3 seeds consisted of an embryo and megagametophyte with a smooth, white and moist surface. In this phase, almost all space within the seed coat was filled (Gruwez et al. 2013).

Data analysis

To quantify the variation in chemical needle composition within and between populations analysis of variance was performed using the aov-function in R 2.15.1 (R Development Core Team 2012).

Linear mixed effects models using the lme-function of the *nlme*-package in R 2.15.1 (R Development Core Team 2012) were applied to determine the relationships between the chemical composition of the needles and environmental variables (temperature, potentially acidifying deposition, precipitation, and the interaction between temperature and depositions) (fixed effect terms). Population was added to the model as random effect term to account for the sampling within populations. Multicollinearity between temperature, potentially acidifying deposition, precipitation was verified by calculating the variance inflation factor (Quinn and Keough 2002).

For each dependent variable, all possible models (i.e. built by each combination of the selected fixed-effects terms) were compared using the Akaike's Information Criterion, adjusted for sample size (AIC_c) (Hurvich and Tsai 1989). The ΔAIC_c of a model was then calculated as the difference between the AIC_c of the model with the best fit and the AIC_c of that model. Models with $\Delta AIC_c \leq 4$ were considered equivalent (Bolker 2008). To determine the relative importance of the explanatory variables, the sum of Akaike weights of the set of all top models ($\Delta AIC_c \leq 4$) in which the variable appeared (Burnham and Anderson 2002) was used. The Aikake weight reflects the weight of evidence in support of a particular model relative to the entire model set, and varies from 0 (no support) to 1 (complete support). For each explanatory variable the relative importance was calculated by summing the Aikake weights of the models containing the variable. Variables with importance values larger than 0.5–0.8 are generally considered to be important to explain variation in the response variable (Calcagno and de Mazancourt 2010; Lindtke et al. 2013; Belaire et al. 2014). An importance value greater than 0.9 is generally considered as 'very important'. Finally, the averaged coefficients of the top models were calculated using the model averaging function based on the AIC_c of the *MuMin* package in R.

To verify whether nitrogen deposition had a similar influence as potentially acidifying depositions, the whole procedure was repeated with nitrogen deposition instead of potentially acidifying depositions as fixed effect term.

To study the influence of the chemical composition of the needles on seed viability after seed phase two and seed phase three, the same method of model selection was used. In this case, generalized linear mixed modelling with binomial distributions was applied, using the *glmmML* function of the *glmmML* package and the *lmer* function of the *lme4* package. This function allows to use binomial distributions since seed viability is expressed as 0 (not viable) or 1 (viable). After testing for multicollinearity between the variables that characterize

Table 1 Mean, minimum and maximum needle concentrations of nitrogen (N), carbon (C), sulphur (S), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and C:N and N:P ratios

Nutrient	Mean (g kg^{-1})	Min (g kg^{-1})	Max (g kg^{-1})	Standard deviation	Coefficient of variation	$F_{19,75}$	P value	R^2
N	14.46	8.29	21.54	2.61	18.05	5.17	< 0.001	0.57
C	507.03	482.52	524.87	9.8	1.93	3.73	< 0.001	0.49
S	1.11	0.56	1.82	0.24	21.62	1.25	0.244	0.24
P	1.22	0.69	1.92	0.28	22.95	4.24	< 0.001	0.52
K	5.76	1.66	10.84	1.58	27.43	7.99	< 0.001	0.64
Ca	10.59	1.51	25.25	4.65	43.91	9.52	< 0.001	0.55
Mg	1.44	0.53	3.02	0.50	34.72	6.67	< 0.001	0.71
C:N	36.34	23.88	61.38	7.46	20.53	7.17	< 0.001	0.70
N:P	12.19	7.31	19.28	2.15	17.64	4.91	< 0.001	0.67

The standard deviation, coefficient of variation and the effects of population (as predictor variable to assess variation between populations) on nutrient concentrations are also given (analysis of variance with population as predictor, F values, P values and the coefficient of determination R^2)

the chemical composition of the needles by calculating the variance inflation factor (Quinn and Keough 2002), only concentrations of N, S, C, P, K, Ca, Mg were selected as fixed effect terms. Again, population was added to the model as random effect term.

Results

Chemical composition of the needles

Needle concentrations were very variable among populations, especially for Ca- and Mg-concentrations (Table 1). Although the among- and within-population variability can be relatively high (Table 1), our values are in agreement with values found in the literature (Fig. 2). Average N and S concentrations were higher than values found in the literature, while Mg concentrations were lower. For K, Mg, C:N and N:P, most variability occurred between populations (Table 1; Fig. 2). Both variability within and between populations was important for N, C, P and Ca (Table 1; Fig. 2). Only for S, variation mostly occurred within populations (Table 1; Fig. 2).

Climatic and atmospheric variables vs foliar chemical composition

Temperature had a strong negative influence on leaf N and P and to a lesser extent on S while C:N was positively affected (Table 2). Potentially acidifying depositions negatively influenced leaf N, P, Ca and positively influenced S and C:N ratios (Table 2). Interactions between temperature and potentially acidifying depositions were important for leaf N, P and C:N. Nitrogen content of the leaves was negatively correlated to potentially acidifying depositions in the cold and moderate cold regions, while in warmer regions, the relationship was positive. The effect of potentially acidifying depositions on P was most pronounced in cold and moderately cold regions. The influence of precipitation was positive on K and C:N and negative on N (Table 2). The effects of N deposition were similar to those of potentially acidifying depositions (results not shown, but see the strong correlation between nitrogen deposition and potentially acidifying deposition in Table S1).

Chemical composition vs seed viability

The viability of SP2-seeds was mostly influenced by leaf K concentrations (negatively) and Mg concentrations (positively). The relationships with the viability of SP3-seeds showed contrasting patterns: leaf N and S had a negative influence on SP3-seed viability while seed viability was positively influenced by leaf P and Ca (Table 3).

Discussion

In this study, we assessed the effects of climate warming and enhanced depositions on the leaf nutrient status of common juniper as a possible pathway to the negative effects on seed viability. Changing precipitation patterns were of minor importance for seed viability. Below, we first discuss how temperature and potentially acidifying depositions may affect the chemical composition of the needles. Second, we clarify how differences in the chemical composition of the needles might explain seed viability.

Temperature and potentially acidifying depositions vs. foliar chemistry

We were able to confirm our first hypothesis that warmer temperatures and enhanced atmospheric depositions have a mostly negative effect on the macronutrient concentrations in the common juniper needles. Leaf nutrient concentrations often reflect the nutrient avail-

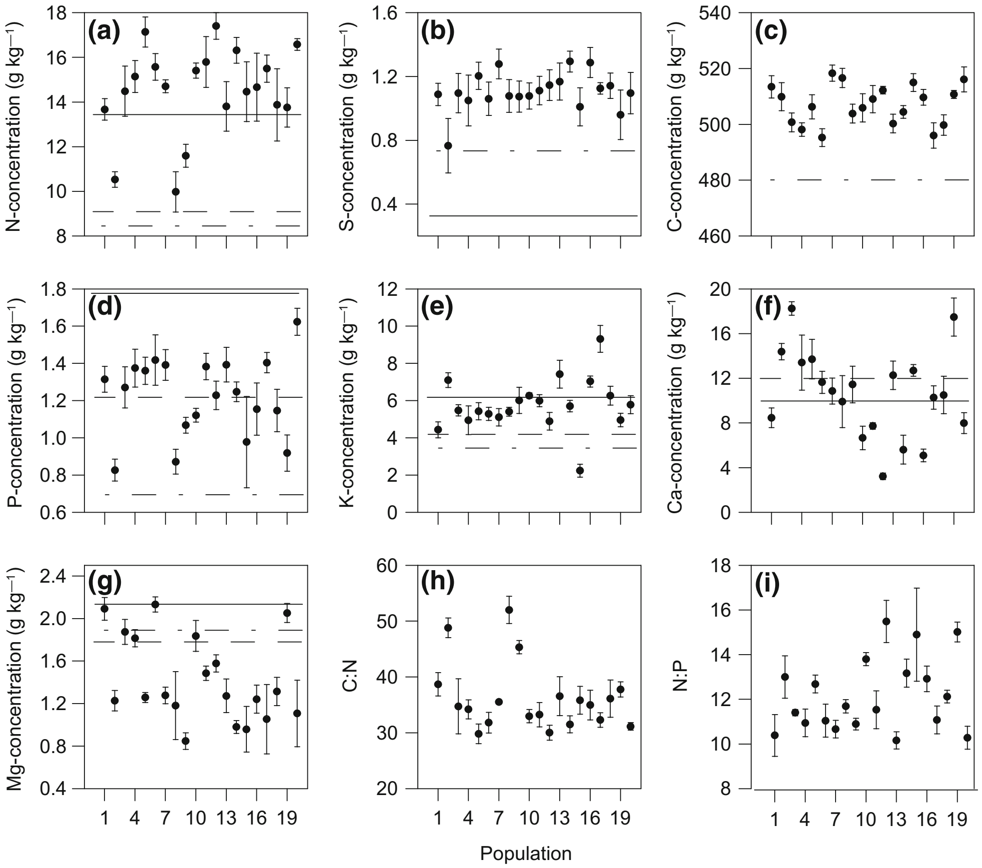

Fig. 2 Average needle concentrations of nitrogen (N), sulphur (S), carbon (C), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), C:N and N:P ratios in the different populations (n = 20 populations). The populations were ordered from the south (*left*) to the north (*right*). To be able to compare our values with those found in the literature, the *full*, *dashed* and *dot-dashed lines* mark the average value for the specific elements found by Rodin and Bazilevich (1967), Henry (1973) and Thomas (2007), respectively. *Error bars* represent standard errors

Table 2 The relative importance and average coefficients of temperature, potentially acidifying depositions, precipitation and the interaction between temperature and potentially acidifying deposition on the needle concentrations of nitrogen (N), carbon (C), sulphur (S), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and ratios of C:N and N:P in the average of the top linear mixed effect models with $\Delta AIC_c \leq 4$ (see "Methods" for more information)

Response	Temperature		Acidifying deposition		Precipitation		Temp. × acid	
	Importance	Coefficient	Importance	Coefficient	Importance	Coefficient	Importance	Coefficient
N	1.00	−3.29E−03	1.00	−4.28E+00	0.84	−1.36E−03	1.00	–
S	0.69	−2.09E−05	1.00	1.74E−01	0.27	1.05E−05	0.23	–
C	0.22	−2.25E−04	0.32	−8.02E−01	0.20	3.45E−04	–	–
P	1.00	−2.87E−04	0.85	−3.42E−01	0.16	−1.06E−06	0.66	–
K	0.32	6.05E−05	0.6	4.98E−01	0.65	9.93E−04	0.04	–
Ca	0.30	1.96E−04	1.00	−4.11E+00	0.48	1.51E−03	–	–
Mg	0.30	−2.73E−05	0.26	−2.60E−02	0.31	−7.13E−05	–	–
C:N	1.00	9.92E−03	1.00	1.16E+01	0.78	3.56E−03	1.00	–
N:P	0.28	9.68E−05	0.30	1.79E−01	0.37	−4.60E−04	–	–

An importance of 1.0 and 0.0 indicate high and low influence, respectively, while "–" means that the variable was not retained in any of the models. An importance value greater than 0.5 is generally considered 'intermediately important'; values greater than 0.9 as 'very important'

ability in the soil (Aerts and Chapin 2000; Hobbie and Gough 2002). Hence, a part of the large variability in leaf nutrient concentrations between populations can be explained by differences in soil conditions. Increasing temperature negatively affected the concentrations of N, P and S in the needles of common juniper, and warming increased the C:N-ratio. The effects of temperature on N were only pronounced in areas with a low acidifying deposition. It is possible that an increase in relative growth rate as a response to higher temperatures and

Table 3 The relative importance and averaged coefficients of the needle concentrations of nitrogen (N), carbon (C), sulphur (S), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), C:N and N:P on the viability of seed phase two (SP2) and seed phase three (SP3) seeds in the average of the top generalized linear mixed modelling with binomial distributions with $\Delta AIC_c \leq 4$ (see "Methods" for more information)

Response	SP2		SP3	
	Importance	Coefficient	Importance	Coefficient
N	0.57	−4.35E−02	1.00	−4.90E−01
S	0.69	−3.58E−01	0.88	−1.07E+00
C	0.21	3.30E−03	0.34	1.89E−02
P	0.22	7.35E−02	1.00	4.73E+00
K	1.00	−2.35E−01	0.66	−3.06E−01
Ca	0.68	3.47E−02	1.00	1.83E−01
Mg	1.00	5.08E−01	0.26	2.06E−01

An importance of 1.0 and 0.0 indicate high and low influence, respectively. An importance value greater than 0.5 is generally considered 'intermediately important'; values greater than 0.9 as 'very important'

CO_2-concentrations is accompanied with a dilution effect on the internal N, P and S pool (e.g. Weih and Karlsson 2001, Doiron et al. 2014). Even though we have no direct growth data available, the negative relationship between temperature and N concentration in the needles in regions with lower acidifying depositions seems to support this dilution effect theory. As mentioned earlier, acidifying depositions are strongly correlated with nitrogen deposition. Thus, in regions that are more N limited, the N pools are insufficiently amplified, to keep up with the possible higher growth rates due to warmer temperatures and higher concentrations of CO_2. Also physiological acclimation can lead to higher N- and P-concentrations in colder regions (Reich and Oleksyn 2004). For example, Hikosaka (1997) found for different plant species that optimal leaf N concentration increased with decreasing temperature.

Similar to other studies (e.g. Innes 1995; Augustin et al. 2005; Thimonier et al. 2010; Sardans et al. 2011), potentially acidifying depositions positively affected needle N-concentrations (only in the warmer regions) and S concentrations. Potentially acidifying depositions consist of N and S particles. Hence, it is not surprising that, due to higher availability, the uptake by the plants increases, leading to higher concentrations in the needles (Augustin et al. 2005). A possible hypothesis to explain the negative effect of deposition on N-concentrations at lower temperatures is that, in colder climates in Europe, N-deposition is typically lower and competition for N, e.g. with micro-organisms, is higher. In addition, mineralisation of N is lower at colder temperatures (Rustad et al. 2001).

Phosphorus and Ca-concentrations were negatively influenced by potentially acidifying depositions. It is possible that potentially acidifying depositions cause soil acidification, which in turn, can decrease the soil Ca-concentrations in the soil and has also an influence on the bioavailability of P in the soil. This mechanism can influence the concentrations in the needles. For example, soil acidification also led to lower Ca-concentrations in the leaves of *Fagus crenata* (Izuta et al. 2004) and N deposition and acidified soils negatively affected Ca- and P-concentrations in *Fagus sylvatica* leaves (Duquesnay et al. 2000). A decreased uptake and increased leaching of cations, including Ca^{2+} caused by potentially acidifying depositions (Bobbink et al. 1992; Schaberg et al. 2001; Krupa 2003) are possible mechanisms to explain the lower Ca-concentrations in the needles.

Foliar chemical composition vs. seed viability

Confirming our second hypothesis, we found that needle nutrient concentrations influenced seed viability of common juniper. Foliar nutrient concentrations can be a good proxy for the nutrient status of the whole plant (e.g. Jett 1987), and thus for the nutrient status of the seeds and their viability. For example, seed weight can be correlated to the needle N, P and K concentrations (Karlsson 2006). Seed mass was positively correlated, and the number of seeds negatively correlated, with needle N concentrations in *Pinus sylvestris* (Savonen and Saarsalmi 1999). Also in e.g. *Malus* sp., the number of flowers was positively correlated with the concentration of N, P and K in the foliage (Marschner 1995). Foliar nutrients can also have a direct effect on the development of seeds; leaf P possibly regulates resource allocation between vegetative and reproductive development (Aerts and Chapin 2000).

Needle Ca- and Mg-concentrations had a positive influence on the seed viability. There are several possible explanations for this effect. For example, both elements are involved in enzyme activity (Raven et al. 1999) and the proportion of Ca-pectate in the cell walls is of importance for fruit ripening in plants (Marschner 1995). If the Ca concentration falls below a critical level in fast growing tissues such as fruits and storage tissues, cell wall stabilization and membrane integrity can be affected (Marschner 1995). Also, low levels of Mg can disturb the export of e.g. carbohydrates from source to sink sites in plants, as the element plays an important role in this process (Marschner 1995).

Phosphorus is not only important for plant viability but also influences seed quality and germination (Bishnoi et al. 2007; Baeten et al. 2010). This is reflected in our results by the positive relation between the needle P-concentration and seed viability. In seeds, P is typically stored as phytate. Phytates are also the main storage sites of K, Mg and, in some cases, Ca and they are involved in the starch synthesis during seed development (Marschner 1995). Hence, P deficiency can restrict seed formation.

Both N and S concentrations had a negative effect on seed viability and the average concentrations were lower than those found in literature (Fig. 2). On the one hand, their influence can also act directly. For example, detoxification, needed in case of a higher uptake of NH_4^+ and NH_3 through canopy exchange, often leads to alterations in the composition of amino acids as plants will choose to store the surplus of nitrogen in compounds with low C:N ratios (e.g. arginine) (Krupa 2003). Among amino acids, arginine is reported to be the most abundant in the female gametophyte of the conifer *Pinus banksiana* (Durzan and Chalupa 1968). Signalling in plants can also be disturbed as arginine acts as an endogenous source of stress-related nitric oxide (NO), a molecular signal that provides the signalling of adaptive structural and functional changes for survival and habituation, but also for damaging reactions, leading to cell death and necrosis (Durzan 2002). On the other hand, if N and S are sufficiently accessible to the plants, a faster growth can lead to deficiencies in other elements including Ca and Mg (Marschner 1995).

Conclusions

The among-population variability in needle nutrient concentrations of common juniper was strikingly high. Both temperature and potentially acidifying depositions significantly influenced the chemical composition of the needles. Changing nutrient availability, leaching and uptake possibly play an important role in the altered nutrient status of the shrubs. In addition, a dilution effect caused by augmented growth can also be of importance. A shortage of P, Mg and Ca, key elements during the nutrient storage throughout the seed development, can lead to anomalies and seed abortion, thereby explaining the low seed viability of common juniper in different regions throughout Europe.

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