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Original article

Needle nutrients in geographically diverse *Pinus sylvestris* L. populations

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Abstract – Nutrient availability differs across climatic gradients, yet the role of genetic variation in potentially adaptive traits related to nutrient acquisition remains poorly understood. We examined needles of diverse Scots pine provenances grown under common-garden conditions throughout their entire life span. Based on similarities in nutrient concentration patterns, two groups of populations were identified. One comprised northern populations from 60° to 56° N, and another included populations from locations between 56° and 49° N. Northern populations sustained significantly higher concentrations of N, P, Ca, Mg, Na, Zn, Cu and Pb. Only K concentration was persistently lower in northern plants. We conclude that intraspecific genetic differences exist in foliage nutrient concentration among diverse populations. Since in northern conditions nutrient availability is often limited as a result of interactions between temperature, litter quality and its mineralization, a tendency toward higher foliage concentrations of macronutrients can be an adaptive feature enhancing plants metabolic activity in their native habitats.

provenance / needle nutrients / climate gradient / seasonal pattern / Scots pine

Résumé – Les nutriments des aiguilles dans le pin sylvestre de différentes origines géographiques. La disponibilité des nutriments varie selon le gradient climatique et le rôle de la variation génétique dans l'adaptation potentielle liée à ce facteur reste mal compris. Nous avons examiné les aiguilles de pin sylvestre de diverses provenances cultivées dans les conditions d'élevage habituel pendant toute la durée de la vie des aiguilles. Nous avons identifié, sur la base de différences dans la concentration des nutriments, deux groupes de populations. La première contient les populations du Nord situées à des latitudes allant de 60° à 56° N. La deuxième est composée de populations originaires de localités comprises entre 56° et 49° N. Les populations du groupe nordique se caractérisent par une concentration significativement plus importante en N, P, Ca, Mg, Na, Zn, Cu et Pb. Seule la concentration en K est plus basse dans les populations nordiques. Nous avons conclu qu'il existe entre ces diverses populations une différence génétique intraspécifique de la concentration en nutriments des aiguilles. La disponibilité de nutriments est souvent limitée au Nord du fait de l'interaction de la température, de la qualité de la litière et de sa minéralisation. Une tendance à une plus grande concentration en macronutriments dans les aiguilles peut avoir un caractère adaptatif aux conditions environnementales dans les habitats naturels de ces plants.

provenance / nutriments de feuilles / gradient du climatique / modèle saisonnier / pin sylvestre

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1. INTRODUCTION

The temporal pattern of changes in foliar nutrient concentration is an important characteristic which can influence canopy CO_2 exchange, growth, dry matter allocation between trees and mycorrhizal fungi, and susceptibility to biotic and abiotic factors [13, 34, 42, 50]. During the life span of foliage, the concentration of nutrients changes depending on their chemical nature, physiological function, supply level and other factors [22, 26, 34]. Trees are characterized by wide genetic variation in nutrient concentration, acquisition and productivity [30, 40, 42]. However, there is a large gap in understanding the scale and mechanisms of intraspecific variation in nutrient behavior in plants, especially in long-lived organisms such as trees [26].

Scots pine (*Pinus sylvestris* L.) is the most widely distributed of the pines and one of the most important timber species in Eurasia. Its natural range extends from Spain in the west (≈ 5° W longitude) to northern Manchuria and the Sea of Okhotsk (130° E) in the east and from 70° N latitude in the northern Scandinavia to 38° N in Turkey. Within this large geographical area mean annual temperatures (*m.a.t.*) range from –10 °C (Yakutiya, Russia) to > 13 °C (southern Europe), and include regions and sites of contrasting fertility and nutrient availability. Therefore, we hypothesize that intraspecific differences in nutrient accumulation and conservation mechanisms may have evolved among diverse populations.

In previous studies we found that common-garden-grown latitudinal populations of *P. sylvestris* and altitudinal populations of *Picea abies* differed in needle N concentration [30, 42]. These results indicated that plants from cold environments have significantly higher foliage N concentration when grown in common conditions, and that this may be an adaptive feature that enhances metabolic activity and growth rates under the low temperatures of their native habitats. However, very little is known regarding the temporal changes in foliage nutrient concentration among diverse populations of trees. To address this issue we studied Scots pine of wide geographic origin, utilizing a common-garden experiment with 16 populations in western Poland. Our study was designed to answer the following questions: Are differences in N concentration among different ecotypes persistent throughout the entire lifetime of needles? Do other nutrients show similar patterns as N? Is it possible based on temporal behavior of nutrients to define biogeographic regions within the species' European range? To what extent does variation in foliage morphology (area and mass growth) and nonstructural carbohydrates affect temporal patterns of nutrient concentration? The broad range of the seed sources and common garden conditions with replicated blocks and plots, uniform soil end environmental factors and frequent observations throughout a three-year period enabled us to address these questions.

2. MATERIALS AND METHODS

2.1. Plant material and study site

Seeds of Scots pine (*Pinus sylvestris* L.) were collected between 1978 and 1980 in 20 locations in Europe as a part of an international collaborative experiment established under the auspices of the International Union of Forestry Research Organizations. Detailed information about this experiment was presented elsewhere [6].

In April 1984, two-year-old seedlings of the 19 populations of Scots pine were planted in a permanent site in the experimental forest, Zwierzyniec, near Kórnik in central Poland (52°15' N and 17°04' E, altitude 70 m). Soil at this site is light sands. Its chemical properties were described in detail in [33]. This site consists of seven blocks. Every provenance was planted in three to seven replicated plots (one per block), 7.2 m × 5.2 m; each with 48 plants (4 rows × 12 plants). The original spacing was 0.6 m within and 1.3 m between rows, and original stocking was 12,834 trees ha^{-1}. In 1994, thinning was conducted and about 60% of trees were removed.

Past studies revealed that populations at this site significantly differ in survival, basal area, aboveground standing biomass, net primary production, CO_2 exchange, genetic structure, growth phenology, foliage nitrogen and nonstructural carbohydrate concentration [27, 29, 31–33, 35–37, 39, 41, 42].

2.2. Environmental conditions

The climate of the region is transitional between maritime and continental. Mean annual precipitation is 526 mm and mean temperature 7.7 °C, with a mean growing season length of 220 days, calculated as the number of days with mean temperature ≥ 5 °C. Meteorological data were obtained from a local meteorological station approximately 2 km from the experimental forest.

This station operates in the state network of meteorological stations in Poland.

The study was conducted over a three-year period, 1996-1998. The studied years differed in climatic patterns. In 1996 mean annual air temperature was 7.1 ºC (0.6 ºC below normal). In contrast, in 1997 and 1998 mean annual temperatures were 9.1 and 9.7 ºC, 1.4, and 2.0 ºC warmer than the long-term average. Mean annual precipitation was 526 mm in 1996, 516 mm in 1997, and 634 mm in 1998.

2.3. Sampling scheme

The ontogenetic pattern of needle nutrient concentration was studied on six geographically diverse populations from the continuous part of the European range of Scots pine in Sweden, Russia, Latvia, Poland, Germany and France (*table I*). Samples of needles of the 1996 cohort were taken 27 times (approximately once per month) beginning May 28, 1996 and ending September 7, 1998. To avoid excessive defoliation and possible confounding effects of crown position [1, 2, 14], twigs were sampled from the sun-lit portion of the crown (between the second and fifth whorl) of different trees on each date. For each sampling date, each provenance was represented by two samples taken from two trees in two different blocks. In addition, in November of 1996 current-year needles of 16 populations from the continuous range of Scots pine in Europe were sampled for nutrient analyses and needle morphology using the same sampling scheme as above.

In order to prevent a possible effect of diurnal variation in needle mass related to carbohydrate accumulation, all needle samples were taken at the same time of day, approximately 4–5 hrs after sunrise. After collection samples were placed on ice in a cooler for transportation to the laboratory (distance of 2 km) for further processing.

2.4. Measurements of nutrient concentration and needle morphology

Nutrients were measured on dried (65 ºC for 48 h) tissue powdered in a Kikro-Feinmühle Culatti mill (IKA Labortechnik Staufen, Germany). For nitrogen analyses

Table I. The origin of seeds of *Pinus sylvestris* used in the study. Provenances are ordered by latitude of origin. Shaded areas are provenances used for studies of seasonal patterns.

Population *No.*	Provenance	Country	Lat. (N)	Long. (E)	Alt. (m)
1	Roshchinskaya Dacha	Russia	60º15’	29º54’	80
15	Sumpberget	Sweden	60º11’	15º52’	185
2	Kondezhskoe	Russia	59º58’	33º30’	70
3	Serebryanskoe	Russia	58º50’	29º07’	80
4	Silene	Latvia	55º45’	26º40’	165
5	Milomlyn	Poland	53º34’	20º00’	110
6	Suprasl	Poland	53º12’	23º22’	160
10	Neuhaus	Germany	53º02’	13º54’	40
11	Betzhorn	Germany	52º30’	10º30’	65
7	Spala	Poland	51º37’	20º12’	160
8	Rychtal	Poland	51º08’	17º55’	190
13	Ardennes	Belgium	50º46’	4º26’	110
12	Lampertheim	Germany	50º00’	10º00’	97
14	Haguenau	France	48º49’	7º47’	150
16	Zahorie	Slovakia	48º46’	17º03’	160
17	Pornoapati	Hungary	47º20’	16º28’	300

the samples were digested by the micro-Kjeldahl method and processed using a BÜCHI Distillation Unit B-322 (BÜCHI Analytical Inc., Switzerland). Analyses of foliar concentrations of P, K, Ca, Mg, Mn, Fe, Cu, Zn, Al, B, Pb, Ni, Cr and Cd were done simultaneously with an Inductively Coupled Plasma Emission Spectrometer (ICP-AES, model ARL 3560) at the University of Minnesota Research Analytical Laboratory, St. Paul, MN, USA (http: //ral.coafes.umn.edu/). The standard dry ashing method of sample preparation for the ICP analysis used in this study may not give complete recovery of Fe, Al and Cr. However, it should not affect relative differences between populations in concentration of these elements and their seasonal changes.

The needle length and projected area was determined using an image analysis system and the WinNEEDLE Software (Regent Instruments Inc., Quebec, Canada). Specific leaf area (*SLA*, defined as the leaf projected area divided by leaf mass) was calculated for the same leaves used for nutrient analyses.

2.5. Measurements of nonstructural carbohydrates

Total nonstructural carbohydrate (*TNC*) concentrations were determined by a modification of the method described by [9, 10]. Sugars were extracted from oven-dried (65 °C, 48 h) tissue powder in methanol-chloroform-water, and tissue residuals were used for starch content determination. Soluble sugars were determined colorimetrically with anthrone reagent at 625 nm within 30 min. Starch in the tissue residual was then gelled and converted to glucose with amyloglucosidase. Glucose concentrations were measured with glucose oxidase by mixing the sample with peroxidase-glucose oxidase-o-dianisidine dihydrochloride reagent. Absorbance was measured at 450 nm after 30 min incubation at 25 °C. Soluble sugars and starch concentration are expressed in percent of tissue dry mass. Soluble carbohydrate concentrations were calculated from standard curve linear regression equations using glucose standard solutions. Data are means of two replications consisting of one composite sample from each of two blocks sampled.

2.6. Statistical analyses

For all variables, statistical differences among provenances and sampling dates were calculated by analysis of variance (GLM procedures). Because different trees and blocks were sampled during the study and samples were pooled by block, the experimental design was considered completely random. Relationships between the sampling day and studied traits were made using correlation and regression analyses. For presentation, both correlation and regression are used but we do not assume that direct causal relations are involved. A Ward's hierarchical clustering method was used to compute cluster groups of Scots pine populations based on 1996-needle-cohort N, P, K, Ca and Mg concentrations on different sampling dates. All statistical analyses were conducted with JMP software (version 3.2.2, SAS Institute, Cary, NC, USA).

3. RESULTS

3.1. Geographic pattern in needle morphology

The data reveal the existence of significant differences in needle morphology among 16 populations grown in common-garden in Kornik, Poland (*table I*). Needle length decreased with increasing latitude of origin from ≈ 9 cm for populations originating from 47 to 54° N latitude to ≈ 7 cm in northern populations (*Nos.* 1, 15, 2, 3 and 4) (*figure 1*). A similar pattern was observed for needle area and mass. Needle width decreased from south to north, ranging from 1.4 to 1.9 mm (*figure 1*). Since needle area and mass were highly correlated with each other ($r^2 = 0.92$, $p < 0.0001$) and changed in parallel with latitude of seed origin, no geographical trends were detected in specific leaf area (*SLA*, *figure 1*). Differences among populations in *SLA* were relatively small and ranged from 26 to 34 $cm^2\ g^{-1}$.

3.2. Lifespan and geographic changes in leaf mineral composition

All concentrations of needle nutrients, except Mg were in an optimal range sufficient for normal growth for *P. sylvestris* (*table II*). Needle Mg concentrations were marginally deficient in central populations (*Nos.* 7, 12 and 14), especially for one-year-old and older needles (*table II*).

The results of the cluster analyses of provenance groups based on similarity of time course of mass-based N, P, K, Ca and Mg concentration in needles (1996 cohort) are summarized in a dendrogram (*figure 2a*). Two distinct groups can be identified, one with northern populations from Sweden, Russia and Latvia (≈ 60 to 56° N) and the other with central European populations from

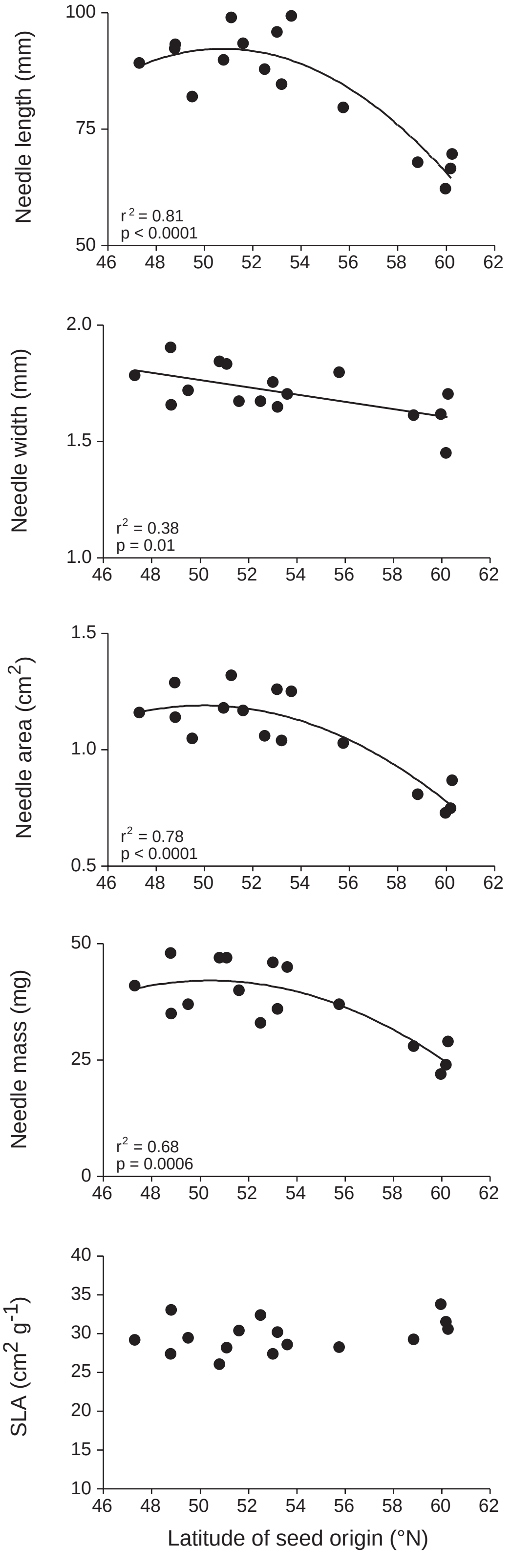


Figure 1. Mean length, width, area, mass and specific leaf area (*SLA*) of current-year needles of Scots pine populations growing in a common garden in Kórnik, Poland (52° N), in relation to latitude of seed origin.

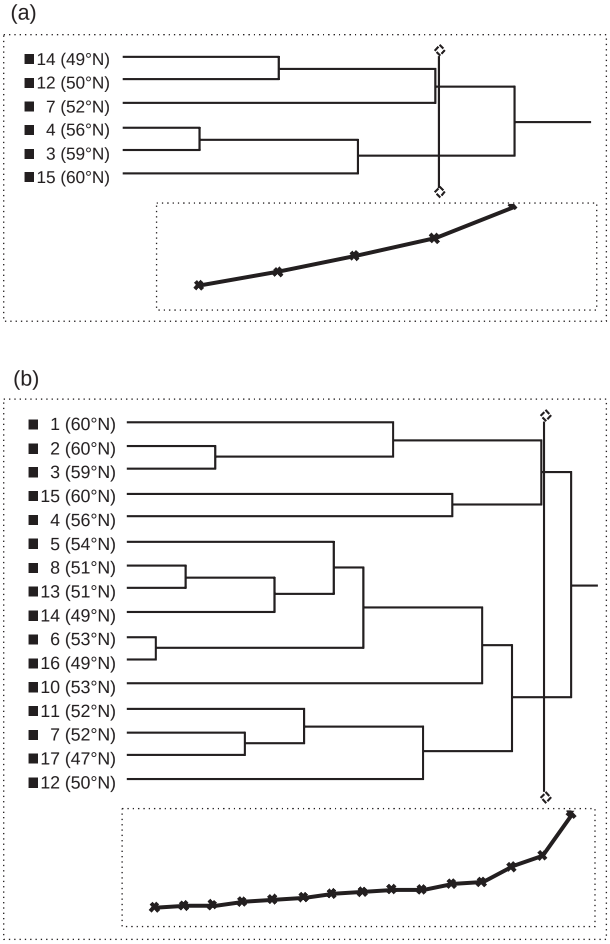


Figure 2. A dendrogram of cluster groupings of provenances of Scots pine based on similarity of mass-based N, P, K, Ca and Mg. As clustering variables for *figure 2a* the concentrations of needle nutrients for different sampling dates were used, and for *figure 2b* November 1996 sampling of current-year needles. The plot beneath the dendrogram presents points for each cluster. The distance and curvature between the points represents the distance between the clusters.

Table II. Element concentrations in foliage of different *Pinus sylvestris* populations for the period spanning the entire life of the 1996 needle cohort (for sampling time $n = 27$). Values are expressed on mass and one-sided projected leaf area. See *table I* for provenance description. The optimal range of mass-based nutrient concentrations are given for one-year-old needles after [18,38].

Element	Units	Population (number and latitude of seed origin)						ANOVA effects $p > F$			Optimal range
		15 (60°N)	3 (59°N)	4 (56°N)	7 (52°N)	12 (50°N)	14 (49°N)	Pop. (P)	Time (T)	$P \times T$	
N	mg g^{-1}	15.9	15.3	14.9	14.8	14.7	14.6	0.002	< 0.0001	0.79	> 14
	g m^{-2}	4.2	4.3	4.3	4.2	4.0	3.9	0.004	< 0.0001	0.89	
P	mg g^{-1}	1.60	1.58	1.52	1.48	1.51	1.56	< 0.0001	< 0.0001	0.40	1.2–1.8
	g m^{-2}	0.41	0.44	0.43	0.40	0.39	0.40	< 0.0001	< 0.0001	0.95	
K	mg g^{-1}	6.03	5.86	5.85	5.95	6.45	6.44	< 0.0001	< 0.0001	0.27	4.5–6.0
	g m^{-2}	1.54	1.62	1.65	1.60	1.69	1.64	0.001	< 0.0001	0.67	
Ca	mg g^{-1}	5.00	4.87	4.82	4.33	4.56	4.97	< 0.0001	< 0.0001	0.40	
	g m^{-2}	1.40	1.49	1.47	1.30	1.34	1.40	0.0003	< 0.0001	0.82	
Mg	µg g^{-1}	861	818	796	713	773	773	< 0.0001	< 0.0001	0.47	800–2200
	g m^{-2}	0.22	0.23	0.23	0.19	0.20	0.20	< 0.0001	< 0.0001	0.98	
Mn	µg g^{-1}	266	240	216	200	234	322	< 0.0001	< 0.0001	0.99	70–400
	g m^{-2}	0.071	0.071	0.065	0.058	0.066	0.088	< 0.0001	< 0.0001	0.97	
Al	µg g^{-1}	186	171	152	126	150	216	< 0.0001	< 0.0001	0.95	
	mg m^{-2}	49.8	50.8	46.0	36.8	42.8	59.7	< 0.0001	< 0.0001	0.88	
Fe	µg g^{-1}	49.1	45.1	48.2	47.4	46.0	47.9	0.0002	< 0.0001	0.01	40–100
	mg m^{-2}	13.7	13.6	14.8	14.3	13.5	13.6	0.0006	< 0.0001	0.29	
Na	µg g^{-1}	27.2	27.7	25.6	26.8	23.3	22.3	< 0.0001	< 0.0001	0.38	
	mg m^{-2}	7.9	8.7	8.1	8.4	7.0	6.3	< 0.0001	< 0.0001	0.51	
Zn	µg g^{-1}	37.1	34.9	32.7	25.3	29.3	31.3	< 0.0001	0.0006	1.00	25–90
	mg m^{-2}	9.9	10.1	9.6	6.9	7.9	8.2	< 0.0001	< 0.0001	1.00	
Cu	µg g^{-1}	3.9	4.1	3.9	3.2	3.8	3.9	0.058	< 0.0001	1.00	3–6
	mg m^{-2}	1.0	1.1	1.1	0.9	1.0	1.0	0.02	0.0257	1.00	
B	µg g^{-1}	19.7	18.7	18.5	18.4	19.4	18.4	0.14	< 0.0001	0.41	8–45
	mg m^{-2}	5.2	5.3	5.4	5.2	5.4	5.0	0.26	< 0.0001	0.92	
Pb	µg g^{-1}	2.8	2.5	2.4	2.4	2.3	2.5	< 0.0001	< 0.0001	0.48	
	mg m^{-2}	0.80	0.75	0.77	0.73	0.69	0.72	0.07	< 0.0001	0.91	
Ni	µg g^{-1}	1.39	1.62	1.22	1.41	1.40	1.59	0.002	< 0.0001	0.52	
	mg m^{-2}	0.35	0.42	0.33	0.36	0.34	0.40	0.0004	< 0.0001	0.23	
Cr	µg g^{-1}	0.44	0.41	0.43	0.41	0.41	0.43	0.26	< 0.0001	0.98	
	mg m^{-2}	0.124	0.124	0.134	0.125	0.118	0.121	0.29	< 0.0001	0.98	
Cd	µg g^{-1}	0.20	0.19	0.17	0.16	0.18	0.21	< 0.0001	< 0.0001	0.30	
	mg m^{-2}	0.054	0.054	0.051	0.045	0.051	0.056	0.02	0.0137	0.66	

Poland, Germany and France (52 to 49° N). Similar groups exist for current-year needles of 16 populations from the continuous European range of Scots pine (*figure 2b*). In the northern group were populations from the area between 60 to 56° N (Russia, Sweden, Latvia) and in the central group were populations from 53 to 47° N in Poland, Germany, Slovakia, Hungary, Belgium and France (*figure 2b*).

The observed differences in mass-based foliage nutrient concentrations among 16 populations were unrelated to *SLA* ($p \geq 0.09$, data not shown). As a result, *SLA* was significantly negatively correlated with area-based P, K, Mg and N (*figure 3*). For the same value of *SLA*, northern populations had 20% higher area-based concentrations of N, 9% higher Mg concentrations, 11% lower K concentrations, and similar concentrations of P. Also several other nutrients showed declines in area-based concentrations with increasing *SLA* (Fe – $r^2 = 0.52$, $p = 0.01$; Na – $r^2 = 0.41$, $p = 0.007$; Zn – $r^2 = 0.29$, $p = 0.03$; Cu – $r^2 = 0.44$, $p = 0.005$; B – $r^2 = 0.61$, $p = 0.0003$).

Correlation coefficients between *SLA* and area- and mass-based nutrient concentration throughout the life span of the needles for two cluster groups are shown in *figure 4*. There were negative correlations between *SLA* and area-based nutrient concentrations of all elements except Ni. Correlations between *SLA* and mass-based nutrient concentrations were positive for elements that decline with needle age and negative for those that increase with needle age (*figure 4*). In general, the correlation coefficients were smaller for elements with strong seasonal variation in concentration (*figure 5*).

For the six populations used in the needle lifespan study, the ANOVA showed a lack of significant sampling date × population interaction for major nutrients (*table II*). However, both groups of populations differed significantly in concentration of N, P, K, Ca, Mg, Na, Zn, Cu and Pb (*table III*, *figure 6*). These differences were significant when expressed both on a mass and area basis. Among the mineral elements, only needle potassium was significantly lower (by 2 to 6%) in the northern population group in comparison with those of central origin. Concentrations of all other elements were significantly higher or similar in needles of northern than central populations. Nitrogen concentration was on average 4 to 8% higher in the northern population group (*table III*). Especially pronounced differences among groups were observed in the autumn, when faster N accumulation was observed in northern populations (*figure 5*). Much larger differences in needle N concentration (> 20%) between northern and central populations for that period of time

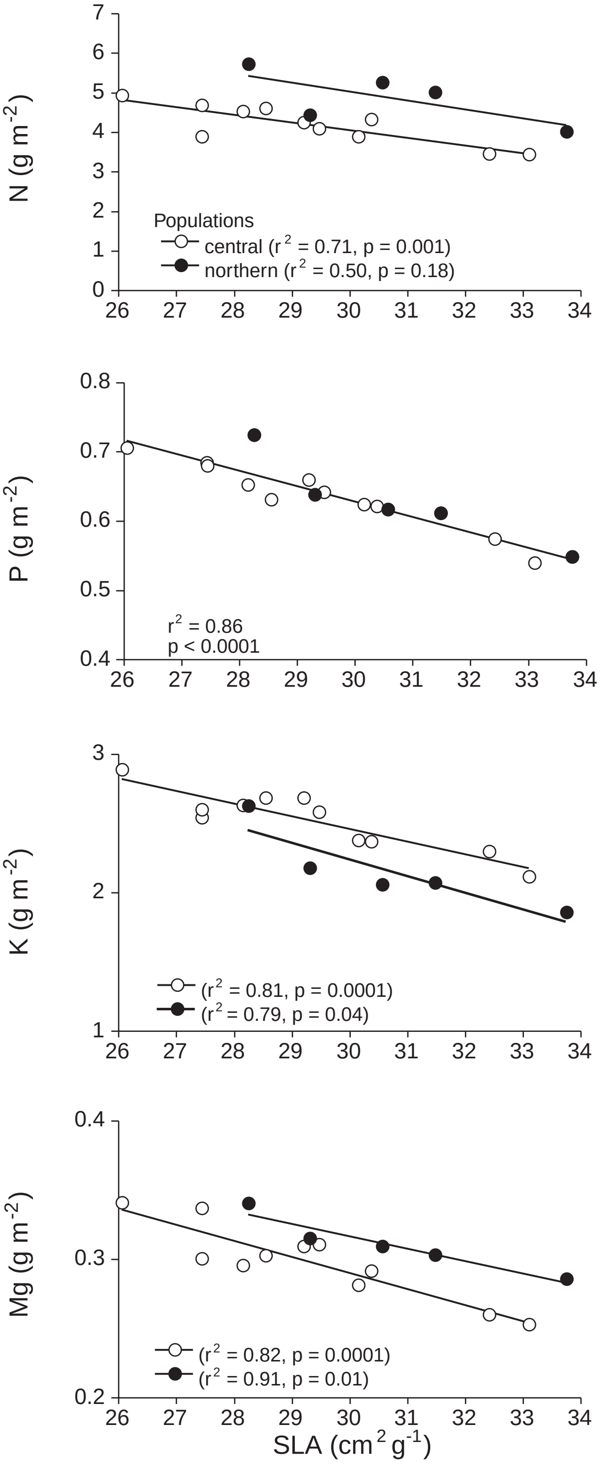


Figure 3. Mean area-based N, P, K and Mg concentrations in current-year needles of 16 Scots pine populations growing in a common garden in Kórnik, Poland (52° N), in relation to specific leaf area (*SLA*).

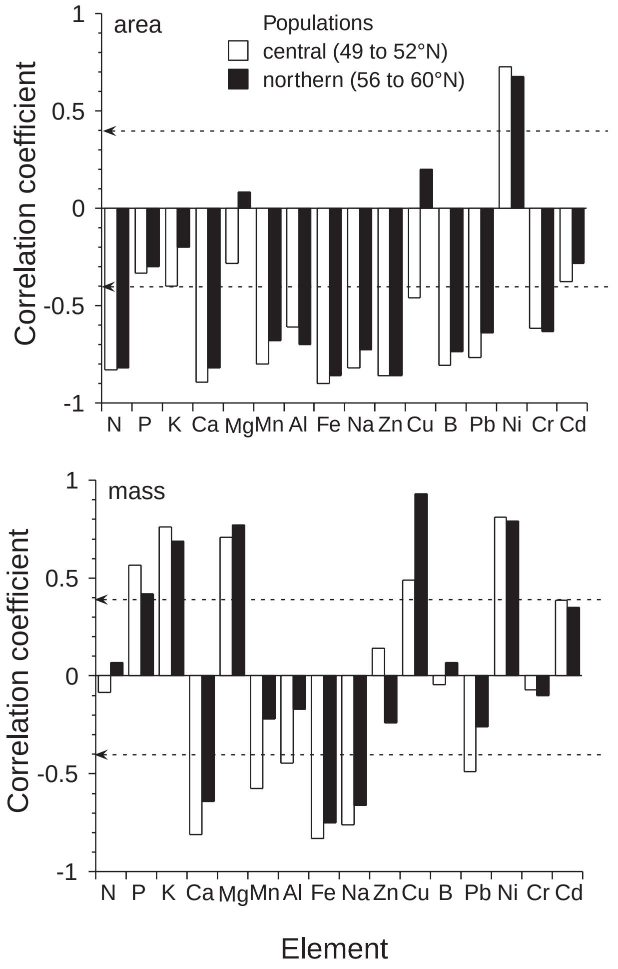


Figure 4. Diagram of correlation coefficients between specific leaf area (*SLA*) and area- and mass-based nutrient concentration for the entire foliage life-span (n = 27 sampling dates).

were observed among the 16 populations sampled in November 1996 (*figure 6*). Similar results were found for current-year needles of 16 populations (*figure 6*) with the exception of Na and Fe concentration. This can be explained by slight seasonal differences in Na and Fe accumulation in needles of the population groups throughout the needle life-span (*figure 5*).

The temporal pattern of nutrient concentrations for the entire needle lifespan is presented in *figure 5*. Mass-based concentrations of N, P, K, Mg, Zn, and Ni were higher in newly formed needles than those in mature or senescing needles (last two data points). Both area– and mass based concentrations of Ca, Mn, Fe, and Na increased nearly continuously with needle age ($r^2 \geq 0.49$, $p < 0.0001$). At the same time mass-based concentrations of P, K, and Mg linearly decreased with foliage age ($r^2 \geq 0.48$, $p < 0.0001$). Concentration of N did not change with needle age when calculated for the entire lifespan of the needles ($r^2 = 0.03$, $p = 0.40$). However, there was a significant declining trend with age when concentration was analyzed only for period when leaves were fully matured (from September 1996 to July 1998, $r^2 = 0.34$, $p = 0.006$). The concentration of Ni decreased within the first year of needle growth and stabilized after that period until the end of the needle life span (*figure 5*).

Both mass- and area-based concentration of Ca increased only during the growing season period and remained unchanged or slightly decreased during the aboveground plant dormancy period. Opposite of the pattern in Ca concentration, P concentration decreased during the growing season and increased in autumn. These fluctuations did not result from changes in nonstructural carbohydrates and were observed on a mass, area and *TNC*-free basis (*figure 7*).

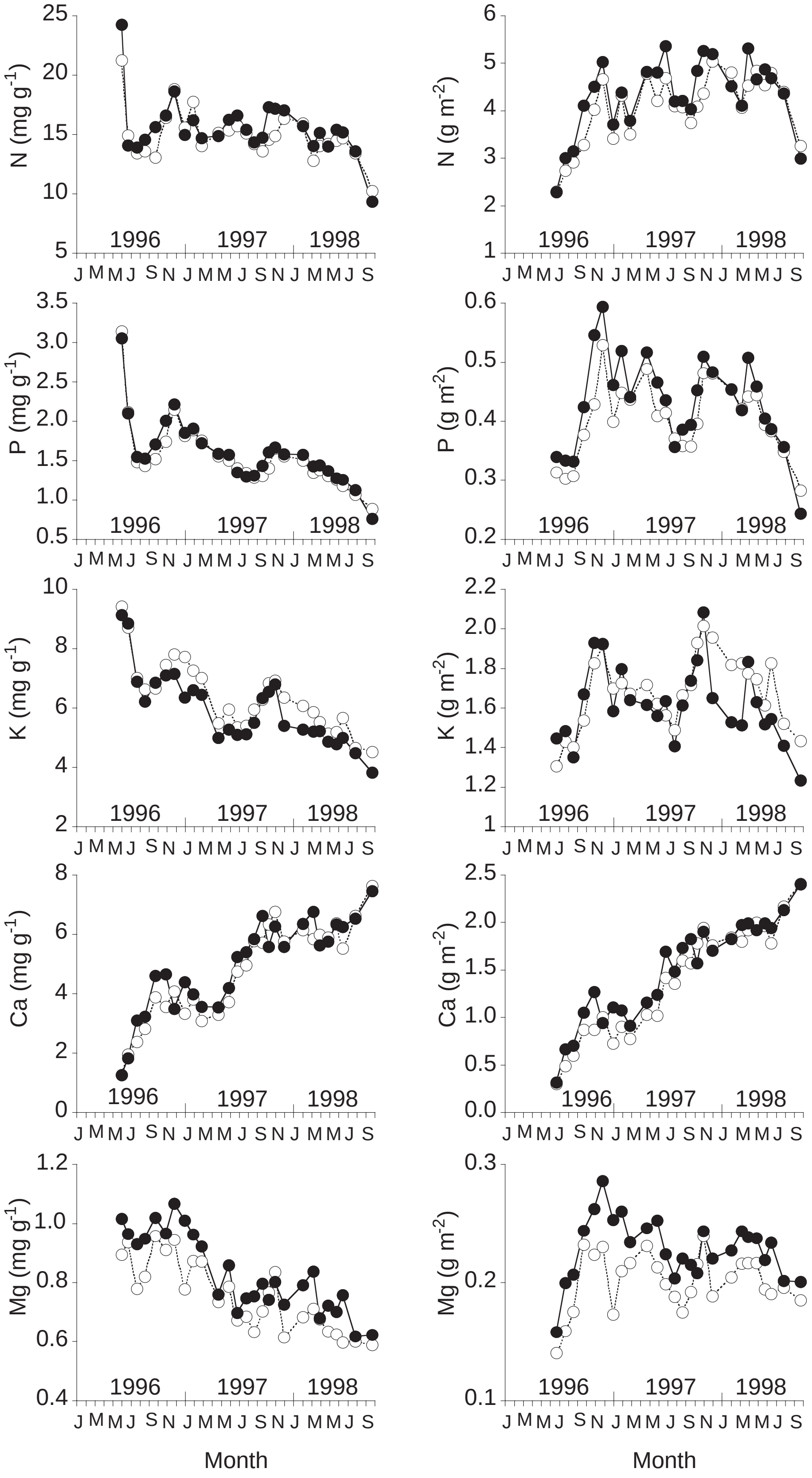
Mass-based concentrations of N, Mg decreased and K, Fe and Na increased with increasing mean annual temperature (*m.a.t.*) of seed origin (*table IV*). Correlation coefficients between nutrient concentrations in the 16 European populations are shown in *table IV*.

4. DISCUSSION

4.1. Differences among populations in nutrient concentration

Little is known about genetic variation in seasonal nutrient concentration patterns in trees. The presented data indicate that Scots pine populations grown in a common garden differ in needle concentrations of all elements except B and Cr. (*table II*). General biogeographic divisions

Figure 5. Seasonal pattern of mass- and area-based concentrations of nutrients for two cluster groups of Scots pine populations growing in a common garden in Kórnik, Poland (52° N). Black points represents northern (56 to 60° N) and open circles central populations (49 to 52° N, *figure 2a*). (See pp. 9–11.)


N (mg g-1)
25
20
15
10
5
N (g m-2)
6
5
4
3
2
1
P (mg g-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
P (g m-2)
0.6
0.5
0.4
0.3
0.2
K (mg g-1)
10
8
6
4
2
K (g m-2)
2.2
2.0
1.8
1.6
1.4
1.2
1
Ca (mg g-1)
8
6
4
2
0
Ca (g m-2)
2.5
2.0
1.5
1.0
0.5
0.0
Mg (mg g-1)
1.2
1.0
0.8
0.6
0.4
Mg (g m-2)
0.3
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1996
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Figure 5.

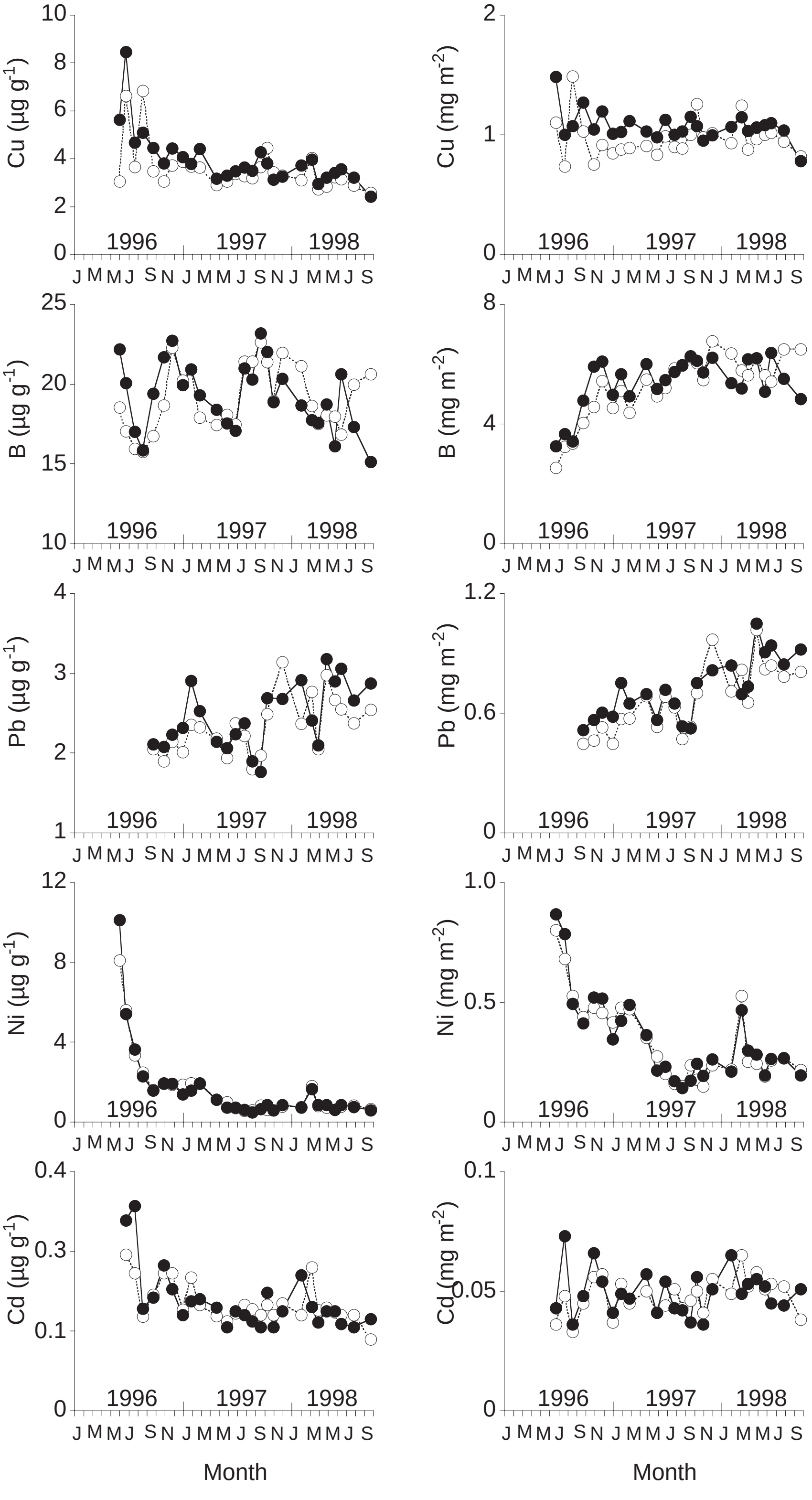

Figure 5. *Continued.*

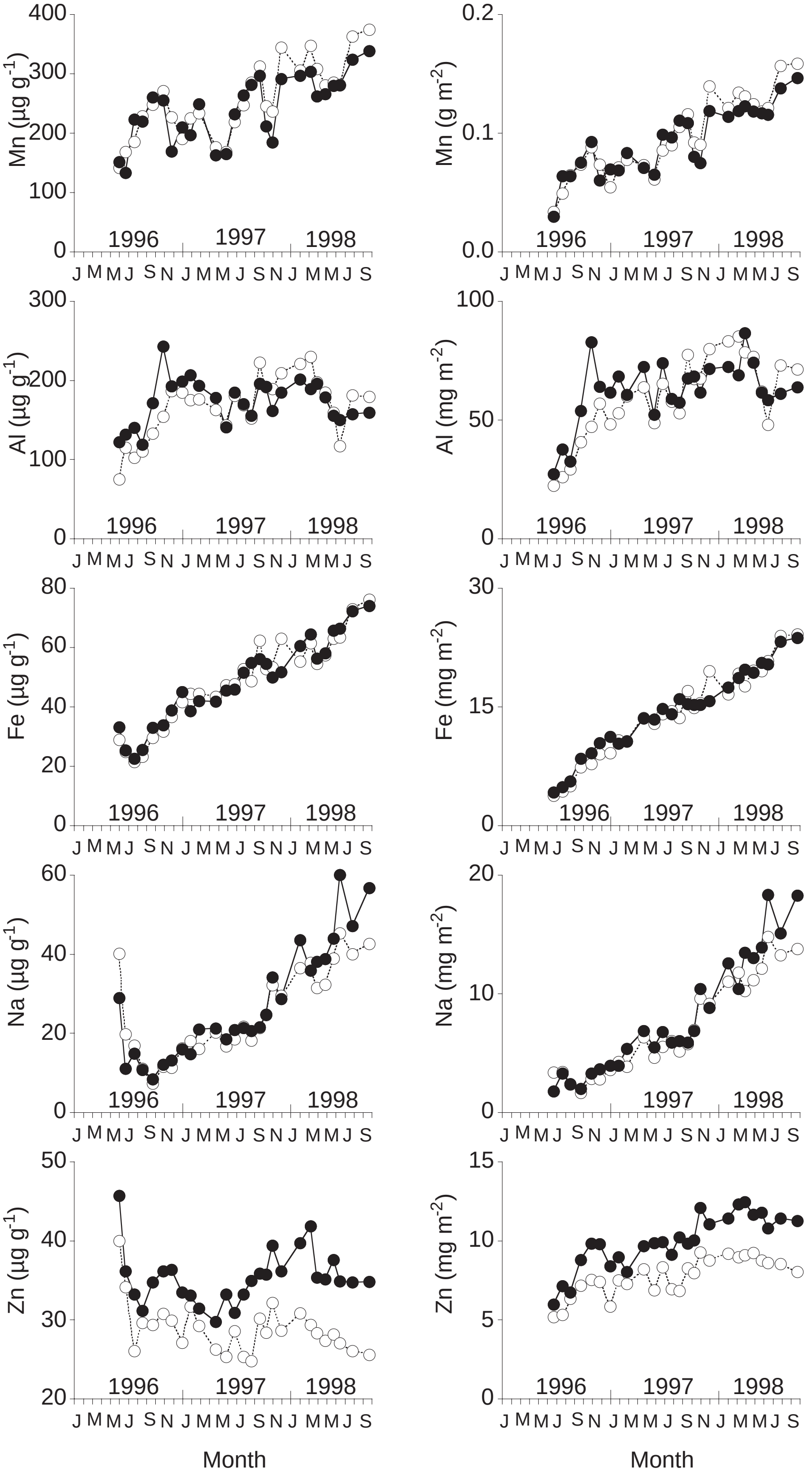

Figure 5. *Continued.*

Table III. Element concentrations in foliage of two groups of *Pinus sylvestris* populations for the period spanning the entire life of the 1996 needle cohort (for sampling time $n = 27$). Values are expressed on mass and one-sided projected leaf areas. See *figure 2* for group descriptions.

Element	Units	Population group		ANOVA effects $p > F$		
		Central (52–49° N)	Northern (60–56° N)	Group (G)	Time (T)	$G \times T$
N	mg g^{-1}	14.7	15.3	0.002	< 0.0001	0.63
	g m^{-2}	4.01	4.34	0.0009	< 0.0001	0.80
P	mg g^{-1}	1.52	1.56	< 0.0001	< 0.0001	0.0006
	g m^{-2}	0.40	0.43	< 0.0001	< 0.0001	0.23
K	mg g^{-1}	6.28	5.92	< 0.0001	< 0.0001	0.03
	g m^{-2}	1.65	1.61	0.01	< 0.0001	0.36
Ca	mg g^{-1}	4.62	4.90	0.007	< 0.0001	0.07
	g m^{-2}	1.35	1.48	0.0005	< 0.0001	0.36
Mg	µg g^{-1}	753	825	< 0.0001	< 0.0001	0.58
	g m^{-2}	0.20	0.23	< 0.0001	< 0.0001	0.73
Mn	µg g^{-1}	252	241	0.08	< 0.0001	1.0
	g m^{-2}	0.071	0.070	0.25	< 0.0001	0.99
Al	µg g^{-1}	164	170	0.32	< 0.0001	0.82
	mg m^{-2}	46.4	49.5	0.23	< 0.0001	0.74
Fe	µg g^{-1}	47.1	47.4	0.65	< 0.0001	0.07
	mg m^{-2}	13.8	14.3	0.24	< 0.0001	0.13
Na	µg g^{-1}	23.9	26.2	0.02	< 0.0001	0.02
	mg m^{-2}	7.1	8.1	0.0008	< 0.0001	0.25
Zn	µg g^{-1}	28.6	34.9	< 0.0001	< 0.0001	0.95
	mg m^{-2}	7.7	9.9	< 0.0001	< 0.0001	0.87
Cu	µg g^{-1}	3.6	4.0	0.04	< 0.0001	0.75
	mg m^{-2}	1.0	1.1	0.02	0.03	0.52
B	µg g^{-1}	18.7	19.0	0.37	< 0.0001	0.004
	mg m^{-2}	5.2	5.4	0.26	< 0.0001	0.037
Pb	µg g^{-1}	2.4	2.5	0.04	< 0.0001	0.64
	mg m^{-2}	0.70	0.75	0.006	< 0.0001	0.73
Ni	µg g^{-1}	1.7	1.7	0.35	< 0.0001	0.24
	mg m^{-2}	0.375	0.358	0.60	< 0.0001	0.95
Cr	µg g^{-1}	0.4	0.4	0.06	< 0.0001	< 0.0001
	mg m^{-2}	0.120	0.125	0.001	< 0.0001	< 0.0001
Cd	µg g^{-1}	0.18	0.4	0.83	< 0.0001	0.41
	mg m^{-2}	0.049	0.050	0.41	< 0.0001	0.40

Table IV. Correlation coefficients for mean annual temperature at place of seed origin and mass-based macro- and microelement concentrations in one-year-old needles of 16 Scots pine provenances in the Kornik, Poland common-garden experiment. Values in bold are significant at $p < 0.05$.

Elt.	Temperature (°C yr^{-1})	N	P	K	Ca	Mg	Mn	Al	Fe	Na	Zn	Cu	B
N	**–0.72**												
P	–0.19	0.47											
K	**0.72**	–0.37	0.14										
Ca	–0.37	0.33	0.08	–0.37									
Mg	**–0.65**	**0.59**	0.42	**–0.50**	**0.50**								
Mn	0.21	0.01	–0.11	–0.13	**0.50**	0.14							
Al	–0.22	0.32	–0.09	**–0.51**	**0.57**	0.32	**0.85**						
Fe	**0.68**	**–0.62**	–0.20	**0.50**	**–0.49**	–0.30	0.15	–0.14					
Na	**0.51**	–0.41	0.01	**0.61**	–0.15	–0.30	–0.08	–0.36	**0.57**				
Zn	–0.38	0.38	**0.72**	–0.29	0.32	**0.60**	0.12	0.28	–0.38	–0.40			
Cu	–0.45	0.36	0.39	–0.46	0.28	**0.68**	0.20	0.38	–0.36	**–0.52**	**0.80**		
B	0.12	0.10	0.23	**0.59**	–0.35	–0.01	**–0.49**	**–0.62**	0.26	**0.62**	–0.27	–0.26	
Ni	0.34	–0.04	0.07	0.12	0.12	–0.18	**0.69**	0.46	0.13	–0.00	–0.05	–0.11	–0.38

of Scots pine populations based on needle N, P, K, Ca and Mg patterns (*figure 2*) are in good agreement with the cluster groupings based on similarities of first-year growth and phenology [28], and shoot growth phenology of 10-year-old trees of the same populations [37]. Growth and physiological differences between studied northern and central European population groups are persistent and were noted in all countries where sites with this experiment were established [7, 32, 48].

Northern populations in this experiment and most other common-garden experiments with Scots pine are characterized by a shorter period of shoot extension, lower biomass and lower aboveground net primary production [6, 32, 36, 37]. This may indicate that lower concentrations of some nutrients in central vs. northern populations can be explained by their dilution through higher biomass growth in the central populations, and consequently a higher nutrient-use efficiency (inverse of concentration) compared to northern populations. Differential concentrations of needle nutrients among these groups (increase of N, P, K, Ca, Mg, Na, Zn, Cu and decrease of K in northern vs. central group) could also reflect genetic differences in mechanisms responsible for their uptake, transport and accumulation. Alternatively, given the apparent phenological constraints on aboveground growth and needle size, higher needle concentrations in northern populations may reflect genetic differences in nutrient storage capacity, especially of mobile elements. At least at the needle-level, differences in nutrient concentration cannot be explained simply by variation in needle morphology. Although needles of northern populations were shorter, narrower, and had lower volume and mass compared to central populations, compared at the same value of *SLA*, some nutrients differed up to 20% between the population groups (*figure 3*). The data showed that Scots pine populations differ consistently in needle N concentration, with populations from colder northern latitudes having greater mass- and area-based N than populations from warmer habitats, when grown in a common environment. The patterns found in this study mirror those in one-year-old seedlings [15] and in 10-year-old trees [42] of the same populations. A comparable pattern of needle N concentration was found also in common-garden grown *Picea abies* populations originated from a broad altitudinal gradient in southern Poland [30]. This finding is consistent with data collected by Körner et al. [16] who notice that peak season N and P concentrations in tissues of plants originating from cold high-altitude sites is higher than those from warmer low-elevation sites. The trend was observed across climatic zones, plant life forms, and species and was not restricted to foliage but also included other plant parts [17].

In northern environments nutrient availability, especially N, is often limited as a result of interactions between low temperature, low mineralization rates, and poor litter quality. Higher foliage concentrations of N, P and Mg in Scots pine populations from cold environments can be adaptive features enhancing their metabolic activity in their native habitats [4, 17].

Averaged for the entire needle lifespan, differences in mass-based foliage N concentration were lower than those expressed on an area-basis (4 vs. 6%) in northern compared to central populations. A similar trend was found by Körner [17] and was related to changes in foliage morphology observed along a temperature/altitude gradient. Leaf morphological traits such as length, width, area and mass decreased with latitude of seed origin (*figure 1*). However, there is no clear latitudinal trend in *SLA* and differences in *SLA* were poor predictors of mass-based foliage nutrients contrary to previously observed trends among plant species representing diverse vegetation types and biomes [43, 44, 45, 46]. Nevertheless, our data indicate that strong negative correlations exist between *SLA* and area-based foliage nutrient concentrations (*figures 3* and *4*).

Northern populations from Sweden, Russia and Latvia exhibited on average 2 to 6% (on area or mass, respectively) lower potassium concentration than populations from the central part or Scots pine European range

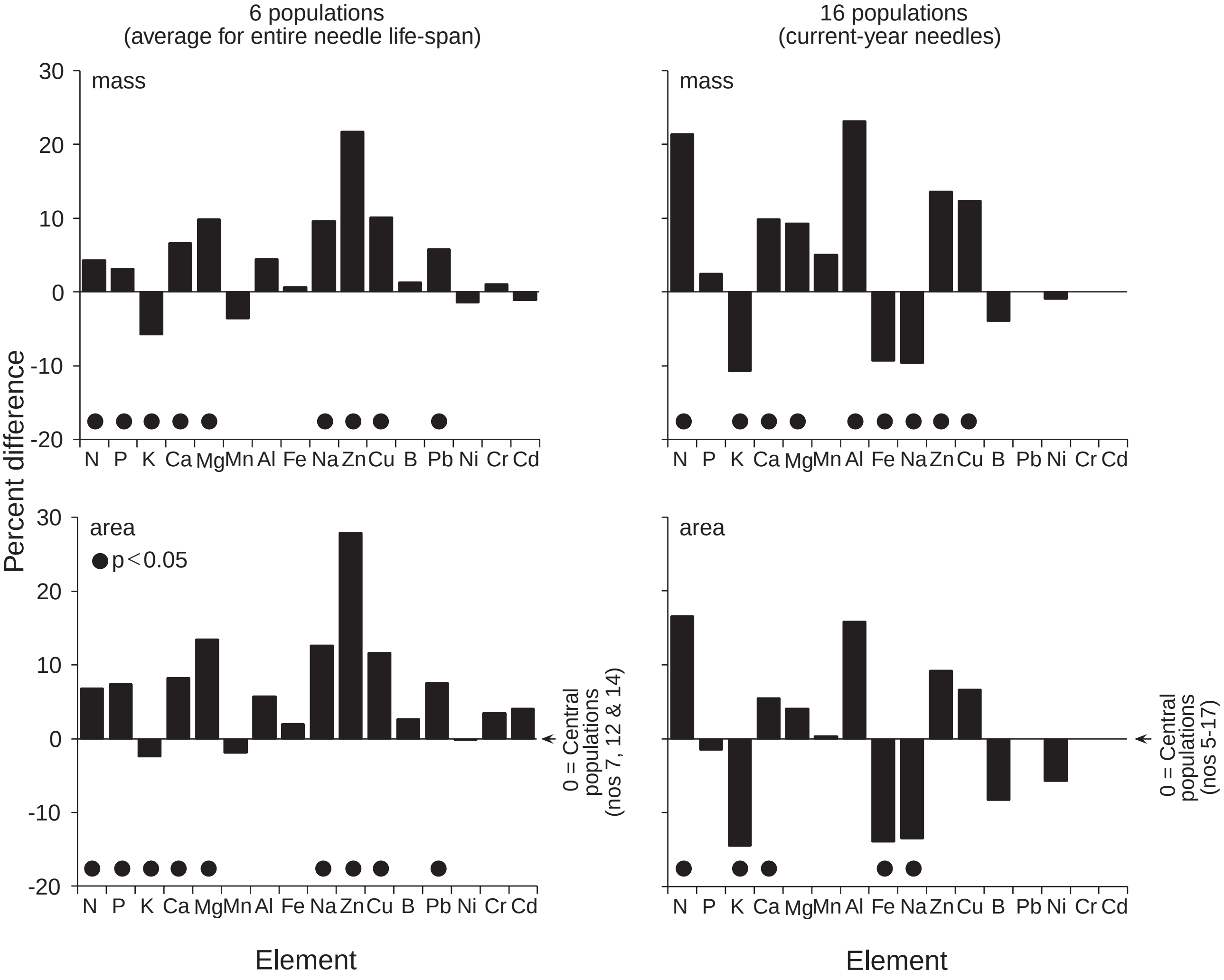

Figure 6. Differences between northern (56 to 60° N) and central (49 to 52° N, *figure 2*) populations of Scots pine in mass- and area based foliage nutrient concentration.

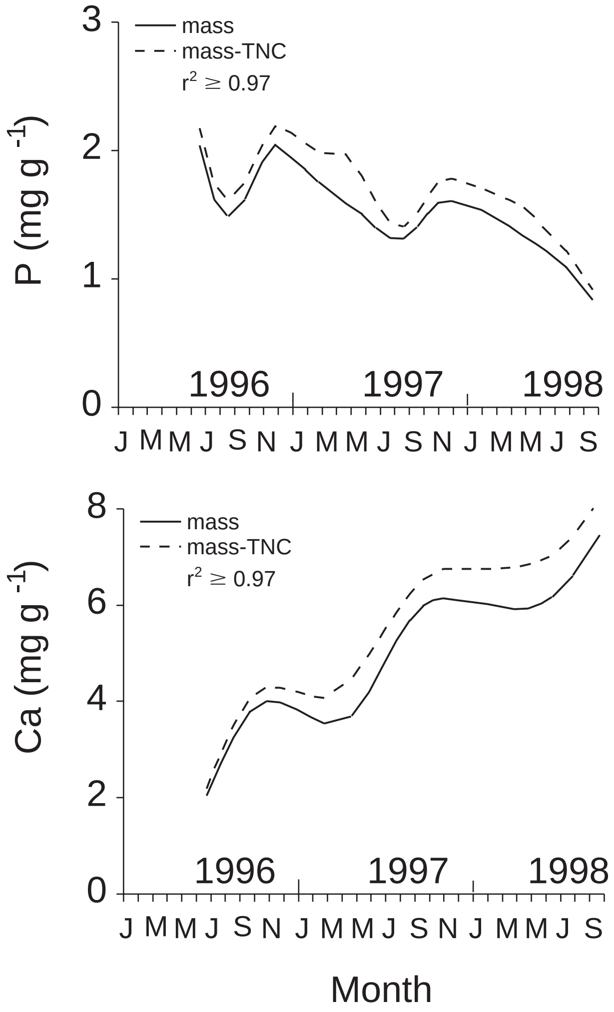

Figure 7. Average seasonal patterns of mass-based concentrations of P and Ca calculated both with and without *TNC*.

in Poland, Germany and France. These differences in K accumulation among population groups are persistent and were observed also in foliage and roots of one-year old seedlings of the studied populations [15] and in most field trials within this experiment (Oleksyn et al., unpublished) and in the some planting sites in the USDA NC-51 regional Scots pine project [47]. Lower concentrations of foliage K are usually observed when the soil water supply is limited [5, 21, 25]. The higher sensitivity of potassium-insufficient plants to drought is related to the role of K^+ in stomatal regulation and the importance of K^+ for the osmotic potential in the vacuoles [23]. However, the water supply hypothesis does not explain observed differences in potassium concentration among studied populations. Microclimatic conditions were uniform for plants of all origins and the aboveground biomass of northern populations in this site is almost 40% lower than those from the central Europe [32]. Therefore, if competition for water resources would play a role in observed foliage K differences then central populations should be lower in that element than those from north.

Northern Scots pine populations are known for their lower water content in needles (in comparison with those of other regions), which is considered an adaptation to cold, dry climates [8, 19]. Most likely a lower K concentration in northern populations is related to their lower foliage water content. The presented results indicate that observed differences are persistent and most likely genetic in nature. It is not clear to what extent the tendency to maintain lower foliage potassium concentrations by northern populations can play a role in their potential drought susceptibility.

4.2. Temporal patterns of needle nutrients

Independent of the origin of populations, mass-based concentrations of mobile elements (N, P, K, Mg) decreased throughout the needle lifespan, with some fluctuations (*figure 5*). Overall, a decline of N concentration was significant only for the period from fully maturated needles to their senescence. Since the concentration of *TNC* and soluble carbohydrates increases with needle age [35], age related declines of mass-based N, P and K concentrations were weaker when calculated on a needle dry mass basis without carbohydrates (data not shown). When concentrations of N, P, K and Mg were expressed on a needle area basis, no age related trends in these elements were observed. Because individual needle mass of Scots pine increases throughout almost the entire lifespan [11, 12, Oleksyn et al., unpublished] observed mass-based trends of these elements are most likely related to a dilution effect.

Over the course of the needle life-span of plants in this experiment, starch concentration changed between 0 and 13% and *TNC* up to 25% of needle dry mass [35]. Thus it is possible that mass-based nutrient concentration of the same population can be lower or higher by as much as ≈ 20–30% due to fluctuation of nonstructural carbohydrates [22, 35, 49].

Seasonal changes of nutrient concentrations in needles were related to the annual phenological cycle and observed both for highly mobile elements such as N, P and K as well as for relatively immobile elements such as Ca, B and Mn (*figure 5*). With the exception of Ca, the lowest concentrations of elements that exhibited seasonal variation in mature needles were observed in spring and early summer, corresponding to the period of intensive growth. Our study provides evidence that a decrease in mature needle nutrient concentrations is not related to changes in needle carbohydrates (*figure 7*, [35]) and most likely can be attributed to the retranslocation of these nutrients to the growing needles, shoots and stems, and to a dilution effect resulting from increasing secondary lignification of needles. Similar decreases in the concentrations of mobile elements were observed in *Pinus sylvestris* [11, 12] and *P. radiata* [3, 24]. In general, concentrations of macro- and micronutrients increase during the period of aboveground organ dormancy in autumn and early winter. The increase in concentration of mobile and semi-mobile elements during that time most likely resulted from resorption from senescing needles. We found that the peak in needle fall is observed between September and December (Oleksyn, personal observation). A similar timing of litter fall was observed in other studies [12, 20].

The discrepancy between mass- and area-based patterns of N, P, K and Mg during needle elongation in spring at the beginning of the needle lifespan indicates that a sharp decline in mass-based nutrient concentration is related to their dilution in increasing needle dry mass and carbon accumulation (*figure 5*).

In summary, we observed distinct differences among diverse Scots pine populations or their groups in nutrient concentration and its seasonal changes. These patterns indicate that nutrient dynamics in Scots pine can be in part under genetic control, and that biogeographic divisions defined by the temporal changes in macronutrients is in good agreement with divisions based on variation growth, phenology and physiological traits. Area vs. mass-based measurements of foliage nutrient concentration may lead to different interpretations of the same data. This may be especially important during the period of intensive spring needle growth. Differences in a majority of foliar nutrient concentrations among diverse populations are persistent throughout the entire lifespan of the needles and vary in predictable fashion.

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