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Forest Ecology and Management 186 (2003) 73–84
Forest Ecology and Management
www.elsevier.com/locate/foreco

Vitality analysis of Scots pines using a multivariate approach

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Received 9 August 2002; received in revised form 7 March 2003; accepted 22 April 2003

Abstract

Multivariate statistical methods were used to analyse the vitality of Scots pines (*Pinus sylvestris* L.) by means of various biochemical, physiological, and nutritional characteristics, irrespective of tree age and without site-specific information. The vitality model was developed in three steps. First, artificial neural networks were used to select a minimal set of biomarkers as input variables with respect to the output variable circular surface increment (regression problem). In the second step, vitality states were classified by using the selected biomarkers (cluster analysis). Finally, discriminant analysis was applied to assign Scots pines to one of four classified vitality states. Sulfate sulfur (SO_4^{2-}-S), non-protein-nitrogen (NPN), arginine (Arg), and chlorophyll*a* (Chl*a*) proved to be the best dynamic input variables to reliably determine the vitality of Scots pines. As the results of regression problem solutions showed, the optimized neural network found growth responses to the driving variables that are valid for both young and mature pine stands. However, the sensitivity analysis of the neural network also indicated that of the four variables, sulfate is the least sensitive. Nevertheless, the sulfate response of the network can be successfully used to analyze the specific effects of multiple exposure on the vitality of Scots pines. To summarize final vitality model enables the vitality of Scots pines to be evaluated without reference to tree age or knowing the specific forest conditions.

Keywords: Biomarkers; Vitality states; Multivariate approach; Scots pine

1. Introduction

With the extensive occurrence of 'novel forest decline' in the early 1980s, forest tree health came in for much public attention and general forest inventories were initiated in many European countries. Visual assessment of defoliation and discoloration became accepted as the standard method for the large-scale monitoring of forest condition in Europe (UN-ECE, 1994). However, 20 years ago it was generally agreed that defoliation and discoloration are unspecific characteristics of tree vitality. Crown density is greatly affected by natural factors, such as tree age, genetic factors, climate, soil conditions, and many abiotic or biotic stresses (Nevalainen and Yli-Kojola, 2000). Yet the results of national forest environmental monitoring programmes, for instance in Norway (Solberg and Tørseth, 1997) and The Netherlands (Erisman et al., 1998; Van der Eerden et al., 1998; Klap et al., 2000), did not provide any evidence of the hypothesized negative effect on crown conditions by sulfur and nitrogen depositions. Despite the clear decreases in sulfur dioxide emissions in eastern Germany, the visible symptoms of needle tip necroses and the needle longevity of Scots pines have remained unchanged (Schulz et al., 1998). Nevertheless tree

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0378-1127/$ – see front matter
doi:10.1016/S0378-1127(03)00236-6

growth has actually increased, especially in formerly highly polluted regions (Neumann and Wenk, 1998). These changes in wood production are inversely correlated with the loss and color of foliage, but correspond to the nutritional status of Scots pine stands over time (Schulz et al., 1998), which is perhaps more important for tree vitality than the above-described crown density characteristics. To resolve this question, we analyzed various biochemical, physiological and nutritional characteristics in needles, including needle tip necroses in young and mature Scots pine stands, along a gradient of sulfur and nitrogen depositions. In contrast to earlier investigations (Schulz, 1989; Schulz et al., 1996, 1999) and corresponding works by other groups (Wild and Schmitt, 1995; Tausz et al., 1998, 2001), in this paper the cross circular area increment was incorporated into multivariate approaches in order to select a minimal set of input variables to produce a vitality-determining output variable. The objective of this study is to evaluate tree vitality, irrespective of tree age and without site specific information, in an objective, repeatable manner we used the term 'vitality' following (Oreshkin et al., 1997) to refer to quantitative assessments of the relative health of trees. Specifically, a Scots pine has good vitality if its current potential of metabolism in 6-month-old needles is sufficient for normal growth. The degree of vitality of a tree is ascertained through the integration of a limited number of biochemical, physiological, and nutritional parameters. Classified vitality states form the basis of the vitality analysis. They stand in relation to multiple exposure at sites and indicate different growth states, such as normal, depressed or accelerated growth according to considerations of Beck (2002). For this top–down approach, various multivariate statistical methods are used. Since neural networks can process information in a non-linear manner (Huntingford and Cox, 1997), they enable completely unconstrained optimisation and can estimate input–output responses without a pre-defined mathematical model. The biomarkers selected by the networks are used for the classification and description of vitality states by means of cluster and discriminant analysis.

Table 1
Site description and site characteristics of studied test plots (scots pine stands)

Site	Test plots (n)	Geographical location	Stand age, 1999 (years)	Soil type	Ecosystem type[a]
Neuglobsow	5	13°02′25″E, 53°09′07″N	69[b]	Dystric Cambisols, sand	Myrtillo-Cultopinetum sylv.
Taura	5	13°00′28″E, 51°28′25″N	50[b]	Cambic Podzols, silty sand	Avenello-Cultopinetum sylv.
Rösa	5	12°29′15″E, 51°37′03″N	65[b]	Spodi-dystric Cambisols, sand	Calamagrostio-Cultopinetum sylv.
Niemegk	1	12°44′16″E, 52°07′06″N	61[b]	Dystric Cambisol, sand	Dicrano-Avenello-Cultopinetum sylv.
Kahlenberg	1	13°49′07″E, 52°53′52″N	54[b]	Dystric Cambisol, sand	Rubo-Avenello-Cultopinetum sylv.
Lichterfelde	1	13°48′19″E, 52°53′40″N	79[b]	Cambic Podzol, silty sand	Calamagrostio-Cultopinetum sylv.
Kienhorst	1	13°29′38″E, 52°56′33″N	67[b]	Ferric Podzol, silty sand	Myrtillo-Cultopinetum sylv.
Wildbahn	1	14°16′43″E, 53°05′43″N	68[b]	Dystric Cambisols, sand	Rubo-Avenello-Cultopinetum sylv.
Neuglobsow	5	13°02′25″E, 53°09′07″N	<40	ND[c]	ND[c]
NSG Plagefenn	5	13°51′20″E, 52°53′14″N	<42	ND[c]	ND[c]
Brieselang	5	12°52′45″E, 52°36′43″N	<38	ND[c]	ND[c]
Gröbern	5	12°27′08″E, 51°41′25″N	<40	ND[c]	ND[c]
NSG Steckby-Löderitz	5	12°00′00″E, 51°54′18″N	<40	ND[c]	ND[c]
NSG Rochauer Heide	5	13°26′48″E, 51°44′14″N	<44	ND[c]	ND[c]
Taubendorfer Heide	5	14°26′46″E, 51°52′00″N	<40	ND[c]	ND[c]
NSG Urwald-Weißwasser	5	14°39′10″E, 51°29′41″N	<42	ND[c]	ND[c]

[a] Typified according to Hofmann (1994).
[b] Mean values of 10 trees.
[c] Not determined.

2. Material and methods

2.1. Study areas and measurements

During the period 1992–2001 more than 100 permanent and temporary test plots in young (25–35-year-old) and mature (60–80-year-old) Scots pine stands were established along a deposition gradient in the lowlands of north–east Germany to provide information for vitality analysis. Details of the different sites are contained in Table 1. At each field site 1–5 test plots were selected in which 10–15 trees were randomly chosen for sampling needles from the youngest age class. In addition, needles were sampled in Nature Park Dübener Heath inside a 2.5 km × 2.5 km grid of 25 test plots. The biochemical, physiological, and nutritional characteristics including needle tip necroses consisted of measurements and estimates taken from mixed needle samples or individual twigs of 15 trees at each test plot. All the needle characteristics are summarized in Table 2 and were determined as described previously (Huhn and Schulz, 1996; Schulz et al., 1998, 1999; Schulz and Härtling, 2001).

To register the mean annual cross-sectional area increment (CSAI) of mature pine stands in the respective observation period, stem disc measurements were performed on 10 trees, that also had been used for needle sampling. The trees were felled in 1999 after the last needle sampling. All stem discs were extracted at breast height (1.3 m). The disks were measured similar to Beck (2001) along four radial lines. Time series of mean $CSAI_t$ were determined by calculating the mean annual CSAI from the cross-sectional area diameters of each year and subtracting two successive cross-sectional area values ($CSAI_t - CSAI_{t-1}$).

Table 2
Mean, standard deviation of the mean (S.D.), minimum and maximum values of biomarkers in 6-month-old needles including stem characteristics of old Scots pine stands ($n = 150$), mean values of young pine stands between brackets ($n = 76$)

Characteristic	Abbreviation (dimension)	Mean	S.D.	Minimum	Maximum
Total nitrogen	N_t (mg g^{-1} dry matter)	17.03 (15.23)	2.41 (1.65)	12.55 (12.20)	24.14 (20.32)
Total phosphor	P_t (mg g^{-1} dry matter)	1.39 (–)	0.22 (–)	0.85 (–)	1.79 (–)
Total sulfur	S_t (mg g^{-1} dry matter)	1.52 (1.42)	0.25 (0.22)	0.93 (1.00)	2.05 (1.87)
Potassium	K (mg g^{-1} dry matter)	4.84 (5.08)	0.35 (0.76)	4.10 (2.83)	5.72 (6.74)
Calcium	Ca (mg g^{-1} dry matter)	2.36 (2.55)	0.36 (0.34)	1.50 (1.19)	3.21 (4.25)
Magnesium	Mg (mg g^{-1} dry matter)	0.77 (0.85)	0.11 (0.13)	0.55 (0.56)	1.14 (1.19)
Sulfate-S	SO_4^{2-}-S (mg g^{-1} dry matter)	0.34 (0.34)	0.13 (0.14)	0.14 (0.08)	0.66 (0.62)
Non-protein-nitrogen	NPN (mg g^{-1} dry matter)	3.98 (3.14)	1.44 (0.42)	2.14 (2.51)	9.16 (5.31)
Phosphate-P	PO_4^{3-}-P (mg g^{-1} dry matter)	0.56 (–)	0.10 (–)	0.38 (–)	0.83 (–)
Glutathione	GSH (mg g^{-1} dry matter)	0.40 (0.38)	0.09 (0.08)	0.21 (0.20)	0.58 (0.57)
Superoxide dismutase (Isozyme 1)	SOD1 (unit mg^{-1} acetone dry powder)	1.27 (1.13)	0.42 (0.34)	0.50 (0.41)	2.30 (1.99)
Glutathione reductase	GLR (unit g^{-1} dry matter)	7.71 (7.43)	2.52 (2.56)	2.74 (3.19)	14.30 (14.33)
Total ascorbic acid	Ascorb (mg g^{-1} dry matter)	3.54 (2.65)	0.59 (0.55)	2.30 (1.50)	4.85 (4.00)
Phosphoenolepyruvate carboxylase	PEPC (unit g^{-1} dry matter)	1.50 (0.98)	0.75 (0.37)	0.42 (0.50)	3.84 (2.45)
Glucose	Gluc (mg g^{-1} dry matter)	3.49 (–)	0.71 (–)	2.04 (–)	5.92 (–)
Glutamine	Gln (mg g^{-1} dry matter)	0.245 (0.137)	0.146 (0.094)	0.032 (0.019)	0.734 (0.562)
Glutamine acid	Glu (mg g^{-1} dry matter)	0.611 (0.665)	0.018 (0.212)	0.216 (0.360)	1.340 (1.405)
Arginine	Arg (mg g^{-1} dry matter)	2.218 (0.314)	3.733 (0.819)	0.003 (0.009)	15.236 (6.887)
Organic phosphor	P_{org} (mg g^{-1} dry matter)	0.83 (–)	0.19 (–)	0.38 (–)	1.20 (–)
Organic sulfur	S_{org} (mg g^{-1} dry matter)	1.18 (1.08)	0.16 (0.14)	0.79 (0.80)	1.52 (1.42)
Protein-N	ProtN (mg g^{-1} dry matter)	13.05 (12.09)	1.43 (1.36)	9.36 (9.29)	15.54 (15.01)
Soluble protein	LProt (mg g^{-1} dry matter)	5.64 (4.35)	3.90 (2.50)	0.58 (0.93)	15.71 (12.40)
Chlorophyll*a*	Chl*a* (mg g^{-1} dry matter)	2.01 (2.00)	0.27 (0.33)	1.38 (1.26)	2.91 (2.76)
Tip necroses of the second needle age class	NK2 (dimensionless)	1.50 (1.22)	0.44 (0.44)	0.63 (0.43)	2.70 (2.70)
Cross-sectional area increment	$CSAI_t$ (cm^2 per area)	5.10 (–)	1.47 (–)	2.82 (–)	9.91 (–)

2.2. Modelling approaches

Initial considerations regarding a multivariate approach for the vitality analysis of Scots pines are to be found in Schulz (1989). In contrast to these preceding investigations, the minimal set of sensitive biomarkers in this work was selected on the basis of artificial neural networks (NN). Irrespective of this, cluster analysis was needed to describe the vitality states of Scots pine trees or stands where the cross-sectional area increments respond similarly to environmental factors as under comparable conditions (Beck, 2002). All calculations were performed using STATISTICA *Neural Networks* or STATISTICA software packages (StatSoft, 1999). We used NN for the selection of variables. The vitality states were classified using the Ward method and were described mathematically using discriminant analysis on the basis of Fisher's discriminant function coefficients (Brosius, 1989).

2.3. Data analysis

Two data sets were used for the modeling approaches. In order to select a minimal set of biomarkers (neural network with regression problem), one data set was used with extensive measurements of biochemical, physiological, and nutritional characteristics including the $CSAI_t$ in 150 test pine stands at the Neuglobsow, Taura, Rösa, and Niemegk sites with 24 independent variables Chl*a*, Ascorb, Gluc, SOD1, PEPC, GLR, S_t, SO_4^{2-}-S, S_{org}, GSH, P_t, PO_4^{3-}-P, Porg, N_t, NPN, ProtN, LProt, Gln, Glu, Arg, K, Ca, Mg, and NK2 with $CSAI_t$ as the dependent variable. For the other two modeling approaches (cluster analysis and discriminant analysis), a second data set with measurements on young and old trees in 226 test plots at various sites was used to group the vitality states using the selected variables and to assign Scots pines to one of the classified vitality states.

In order to create and test the effectiveness of the NN for the feature selection, we used the Advanced Intelligent Problem Solver (AIPS) within STATISTICA *Neural Network*. The AIPS uses the backpropagation algorithm to solve this problem. Generally, multilayer perceptron networks consisting of three layers were favored for feature selection. To control the selection process more closely, the data set ($n = 150$) was divided into training and verification subsets. A test subset was not used due to the small number of cases. The cases were divided in the proportion 2:1 between the training and verification sets. Training was based on an iterative gradient algorithm designed to minimize the mean square error between the actual output and the desired target by using a minimum improvement level (training and verification error do not improve by at least the amount given over a set number of epochs). All optimizations finished after 100 epochs. The results of different variable combinations were evaluated using the independent verification set. Only NN with high cross-verified performance were selected. The best of many models was chosen on the basis of the lowest root mean square error (RMSE) and the highest explained variance (R^2). The classification and assignment problems were solved by means algorithms of cluster and discriminant analysis as described previously by Schulz et al., 1999, 2000.

3. Results and discussion

3.1. Biochemical, physiological, and nutritional characteristics of Scots pines needles and cross-sectional area increment

A summary of biochemical, physiological, and nutritional properties as input and output variables for the vitality evaluation of Scots pines (*Pinus sylvestris* L.) is presented in Table 2. The table contains the mean, standard deviation (S.D.), minimum, and maximum values for needles and stems of young and mature trees. The concentrations of the macro-nutritional elements N_t, P_t, S_t, K, Ca, and Mg in 6-month-old needles of both data sets are in the range of values reported by Krauß (1990) and Prietzel and Kölling (1998), and are mainly influenced by atmospheric sulfur and/or nitrogen deposition and less by ozone as already previously reported (Schulz et al., 1998; Schulz and Härtling, 2001). Although some responses, such as pools of P, K, and Ca, follow the deposition gradient, the sulfur and nitrogen metabolism only is affected by the impact of pollution (Schulz et al., 1998). Therefore, it is not surprising that the concentrations of the sulfur- and nitrogen-containing metabolites, such as SO_4^{2-}-S, GSH, NPN, Gln, Glu, Arg, and LPROT as well as the corresponding enzymes GLR and PEPC indicate a broad range between the minimum and maximum values of the parameters. Additionally, it is particularly interesting that the range of most parameters is independent of tree age. For instance, there are no differences between the concentrations of SO_4^{2-}-S and Chl*a* in needles of young and mature trees. Generally this observation leads to the assumption that the data sets of young as well as of mature trees can be used for the multivariate approaches to evaluate the vitality of Scots pines.

3.2. Input variable selection

Neural networks (NN) were used to select a minimal set of biomarkers. The following approaches were adopted to solve this non-linear regression problem with NN. At first, feature selection algorithms were run to explicitly identify input variables that do not contribute to the performance of the networks, and to remove them. In the next step sensitivity analyses were started using the selected variables in combination with other ones to ascertain which input variables are considered most important by that particular NN. All calculations were performed by means of the data set of mature Scots pines with 150 observations on 24 input variables (Chl*a*, Ascorb, Gluc, SOD1, PEPC, GLR, S_t, SO_4^{2-}-S, S_{org}, GSH, P_t, PO_4^{3-}-P, P_{org}, N_t, NPN, ProtN, LProt, Gln, Glu, Arg, K, Ca, Mg, and NK2) and one output variable ($CSAI_t$). For input feature selection we used the best trained NN and selected a combination of genetic and backward stepwise algorithms. Irrespective of the composition of variables (e.g. with or without N_t, S_t, and P_t), a consistent ordering of the variables was obtained. NPN, Arg, and PEPC contributed to the performance of the NN. The characteristics N_t, P_t, and Chl*a* had insignificantly smaller contributions, while the SO_4^{2-}-S as well as S_t were removed first from all inputs. These results were particularly surprising, because Scots pines are known to be influenced by SO_2 deposition (Schulz et al., 1999), and so it had been assumed that SO_4^{2-}-S indicates a strong predictive variable for growth. On the other hand, the model may suffer from the number of variables, because it is difficult for the algorithm to be sufficiently sensitive, especially if the set only contains a few weakly predictive variables, such as K, Ca, Mg, or Gluc. By comparison, the selected variables NPN, Arg, and PEPC, as well as important combinations, e.g. with N_t, S_t, SO_4^{2-}-S, P_t, or Chl*a*, can be verified by comparing the root mean square error (RMSE) and the explained variance (R^2) generated by sensitivity analysis. The validation results of the most important combination of variables are shown in Table 3. The optimum construction for NN models presented here uses the variables Arg, NPN, and Chl*a* in combination with N_t, S_t, SO_4^{2-}-S, P_t, or PEPC (see models 5–9). The best fit values were obtained when using N_t or SO_4^{2-}-S as an extra input variable (see models 5 and 7) where only one hidden neurone was necessary. Using, the variables S_t, P_t, or PEPC as an extra input for these sensitivity analyses (see models 6, 8 and 9) only negligibly improved model performance, but increased the number of hidden neurones. All other combinations of variables in the order Arg, NPN, N_t, or Arg, NPN, PEPC with S_t, SO_4^{2-}-S, P_t or PEPC as an extra input variable led to a decrease in model fit for the output variable $CSAI_t$ (see models 1–4 and 10–12). We then analyzed model 5 with the inputs Arg, NPN, Chl*a*, and N_t including model 7 with the inputs Arg, NPN, Chl*a* and SO_4^{2-}-S. Although in model 5 the RMS error of the variable N_t (0.60) was higher than that of SO_4^{2-}-S (0.58) in model 7, indicating that the network performance deteriorates if N_t is replaced by SO_4^{2-}-S, the combination of variables in the order Arg, NPN, Chl*a* and SO_4^{2-}-S was favored instead of the construction with N_t as an input variable. Finally, this combination

Table 3
Model misfits (RMSE) of neural networks using different sets of input variables, explained variance between brackets

Model	Number of hidden nodes	Input variables	Output variable ($CSAI_t$)
1	2	Arg, NPN, N_t, S_t	0.73 (0.79)
2	1	Arg, NPN, N_t, SO_4^{2-}-S	0.72 (0.81)
3	13	Arg, NPN, N_t, P_t	0.73 (0.79)
4	1	Arg, NPN, N_t, PEPC	0.72 (0.81)
5	1	Arg, NPN, Chl*a*, N_t	0.57 (0.88)
6	8	Arg, NPN, Chl*a*, S_t	0.58 (0.86)
7	1	Arg, NPN, Chl*a*, SO_4^{2-}-S	0.57 (0.88)
8	8	Arg, NPN, Chl*a*, P_t	0.56 (0.88)
9	8	Arg, NPN, Chl*a*, PEPC	0.56 (0.88)
10	2	Arg, NPN, PEPC, SO_4^{2-}-S	0.78 (0.77)
11	2	Arg, NPN, PEPC, S_t	0.78 (0.77)
12	2	Arg, NPN, PEPC, P_t	0.78 (0.77)

of variables was chosen to prevent model artefacts in further multivariate approaches (cluster analysis, discriminant analysis) due to the close correlation between the nitrogen-containing input variables (Huhn and Schulz, 1996) and to ensure that the effect of SO_2 on tree vitality is considered (Schulz et al., 1999).

3.3. Cluster analysis

Cluster analyses were performed using the quadratic Euclidean distance measure and the Ward criterion (Brosius, 1989). These were used to address the following question: How can the total of 226 measuring pine stands be grouped into clusters in which the vitality of Scots pine is described by a number of selected biomarkers in relation to the multiple exposure at the sites indicating different states of growth.

To answer this question, cluster analysis was performed based on the parameters of the four biomarkers selected from the NN as stated earlier. The results are summarized in Table 4 and reveal four clusters with distinct average concentrations of the four needle characteristics. Examination of the average concentration values leads to interpretation in terms of the vitality states listed in the first column, which is now demonstrated for the cluster representing mainly 'depressed growth'. As can be seen from the table, both clusters contain the lowest average concentrations of NPN (3.04 ± 0.29 or 3.15 ± 0.37 mg g^{-1} dry matter), Arg (0.15 ± 0.32 or 0.32 ± 0.40 mg g^{-1} dry matter) and Chl*a* (1.81 ± 0.23 or 1.87 ± 0.18 mg g^{-1} dry matter), but significantly different mean concentrations of SO_4^{2-}-S (0.22 ± 0.06 or 0.49 ± 0.06 mg g^{-1} dry matter), which in turn also indicates the lowest growth with values of $CSAI_t$ (3.89 ± 0.58 or 4.03 ± 0.56 cm^2 per year) compared to the vitality states 'normal growth' (5.40 ± 0.63 cm^2 per year) and 'accelerated growth' (7.60 ± 1.23 cm^2 per year). The other characterizations of clusters in terms of vitality states were performed in a similar way following the specific composition profiles and maximum average concentrations of compounds as outlined before. Two of the four clusters listed in Table 4 represent the previously defined vitality states 'normal growth' and 'accelerated growth', while in addition there are two clusters are assigned to different levels of 'depressed growth'. The vitality states 'normal growth' and 'accelerated growth' are characterized by more or less normal mean concentrations of SO_4^{2-}-S (0.34 ± 0.08 or 0.41 ± 0.13 mg g^{-1} dry matter) in 6-month-old needles, but differ greatly as regards their mean NPN (3.63 ± 0.46 or 6.86 ± 1.17 mg g^{-1} dry matter) and Arg (0.99 ± 1.28 or 9.84 ± 2.99 mg g^{-1} dry matter) concentrations. For Chl*a*, 'normal growth' and 'accelerated growth' yield significantly higher concentrations (2.29 ± 0.21 or 2.10 ± 0.25 mg g^{-1} dry matter) than 'depressed growth' (1.81 ± 0.23 or 1.87 ± 0.18 mg g^{-1} dry matter). The assignment of individual sites to clusters is not shown.

Now the vitality states need to be interpreted at a biochemical-physiological and nutritional level. Generally speaking, the individual vitality states are the result of the different sulfur and nitrogen nutrition of Scots pines due to the multiple exposure patterns of airborne sulfur and nitrogen compounds in forest ecosystems. When plotted against $CSAI_t$, the SO_4^{2-}-S and NPN values reflect four patterns of variation of the cross-sectional area increment (Fig. 1a). As can be seen from the three-dimensional graph, the white circles reflect growth at low S nutri-

Table 4
Means of vitality states ± standard deviations based on the cluster analysis with four selected needle characteristics and a random sample with 226 cases (76 young and 150 old pine stands)

Vitality state	Needle characteristics							
	SO_4^{2-}-S (mg g^{-1} dry matter)	NPN (mg g^{-1} dry matter)	Arg (mg g^{-1} dry matter)	Chl*a* (mg g^{-1} dry matter)	$CSAI_t$ (cm^2 per area)	ProtN (mg g^{-1} dry matter)	S_t:N_t (g mole/g mole)	S_{org}:N_t (g mole:g mole)
Depressed growth 1 ($n = 77$)	0.22 ± 0.06(b)	3.04 ± 0.29(b)	0.15 ± 0.32(b)	1.81 ± 0.23(b)	3.89 ± 0.58(b)	11.53 ± 1.18(b)	0.037 ± 0.003(b)	0.030 ± 0.002(b)
Depressed growth 2 ($n = 55$)	0.49 ± 0.06(b)	3.15 ± 0.37(b)	0.32 ± 0.40(b)	1.87 ± 0.18(b)	4.03 ± 0.56(b)	12.46 ± 1.26(b)	0.045 ± 0.003(b)	0.031 ± 0.002(a)
Normal growth ($n = 71$)	0.34 ± 0.08(a)	3.63 ± 0.46(a)	0.99 ± 1.28(a)	2.29 ± 0.21(a)	5.40 ± 0.63(a)	13.67 ± 1.02(a)	0.040 ± 0.003(a)	0.032 ± 0.002(a)
Accelerated growth ($n = 23$)	0.41 ± 0.13(a)	6.86 ± 1.17(b)	9.84 ± 2.99(b)	2.10 ± 0.25(b)	7.60 ± 1.23(b)	13.99 ± 0.63(a)	0.036 ± 0.004(b)	0.028 ± 0.002(b)

SO_4^{2-}-S–sulfate sulfur, NPN–none-protein nitrogen, Arg–arginine, Chl*a*–chlorophyll*a*. In addition, group means ± standard deviations of other needle characteristics (S_t:N_t–total sulfur/total nitrogen, S_{org}:N_t–organic sulfur/total nitrogen, ProtN–protein-N, $CSAI_t$–cross-sectional area increment, values of young pine stands were calculated with model 7 of neural network in Table 3). Significant differences were determined according to the Mann–Whitney *U*-test. Different alphabets (a and b) within the columns indicate significant differences at $P < 0.05$ between the vitality states (reference vitality state: normal growth).

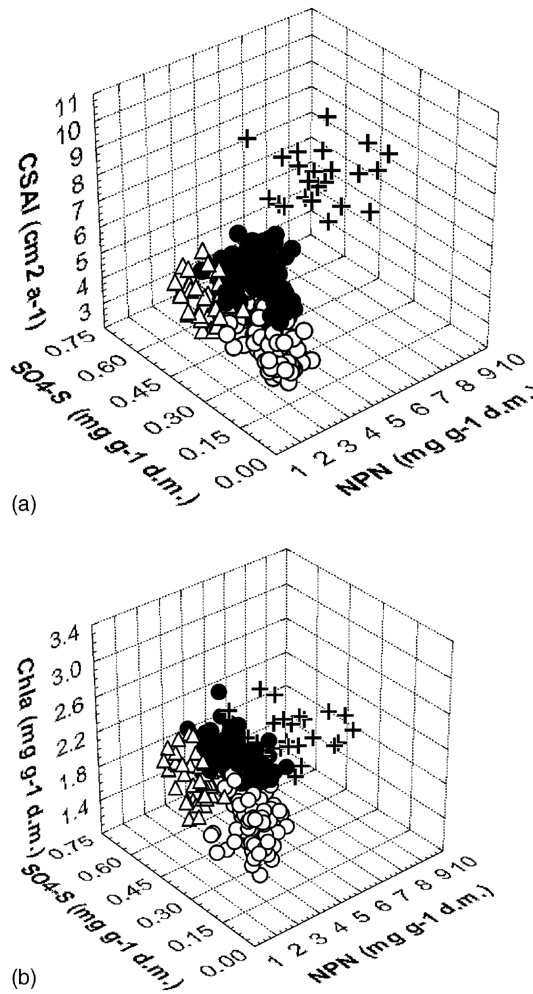

Fig. 1. Graphic display of the four vitality states in the three-dimensional space. The symbols white circles (○) characterize depressed growth 1, white triangles (△) depressed growth 2, black circles (●) normal growth and crosses (+) accelerated growth. (a) Cross sectional area increment (CSAI) as function of sulfate sulfur (SO_4^{2-}-S) and non-protein-N (NPN) (b) Chlorophyll*a* (Chl*a*) as function of sulfate sulfur (SO_4^{2-}-S) and non-protein-N (NPN).

tion, the triangles growth at high S nutrition, and the black circles growth at normal S nutrition, whereas the crosses represent growth at normal S nutrition, but increased N nutrition. The same patterns are obtained if the chlorophyll*a* concentration is considered depending on the sulfate sulfur and the NPN concentration in three-dimensional space (Fig. 1b). On the basis of these observations it is assumed that the vitality of the Scots pines investigated was mainly affected by imbalances in the S and N nutrition. This conclusion is confirmed by the functional relations between the molar sulfur:nitrogen (S_t:N_t) ratio and SO_4^{2-}-S or NPN, respectively. As can be seen from the plots in Fig. 2a and b, the mean value of S_t:N_t ratio on a gram-mole basis of the vitality state 'normal growth' is 0.040 ± 0.003. This mean value is consistent with the calculated mean value of 0.032 ± 0.002 for S_{org}:N_t ratio on a gram-mole basis of the vitality state 'normal growth' (Table 4), which is in line with the investigations by Malcolm and Garforth (1977), and indicates a balanced S:N nutrition for the optimum protein synthesis of *P. sylvestris* L. All deviations from the threshold value (0.040 ± 0.003) or line in Fig. 2a and b indicate imbalances in S and N nutrition, which are mainly reflected by the vitality states 'depressed growth' with low or high SO_4^{2-}-S concentrations as well as the vitality states 'accelerated growth' with normal S status, but an accumulation of NPN. As previously reported (Huhn and Schulz, 1996), this soluble nitrogen fraction indicates the massive enrichment of the amino acid arginine if the NPN concentration exceeds the maximum value of 5 mg g^{-1} dry matter (Fig. 3). Simultaneously the concentration of chlorophyll*a* decreases due to increased protein synthesis. Therefore, the accumulation of arginine can be designated as an early indication of disturbance to N metabolism. The mean value of 3.63 ± 0.46 mg g^{-1} dry matter reflects a threshold for normal growth-dynamics with mean chlorophyll*a* concentrations of 2.29 ± 0.21 mg g^{-1} dry matter. This mean value of chlorophyll*a* corresponds to 2.90 ± 0.27 mg total chlorophyll g^{-1} dry matter, which according to Wild (1987) represents vital conifers. The next question is whether vitality states can be described by a system of mathematical functions that allow new cases or needle samples to be allocated to the existing cluster scheme.

3.4. Discriminant analysis

In the previous section, a cluster scheme was derived that allows the systematic features to be unravelled behind the biomarker patterns as characterized by the total of 226 needle samples of young and mature pine stands: four clusters representing different vitality states, and clusters indicating various growth levels depending on environmental impacts (Table 4). A mathematical means to test the significance of these

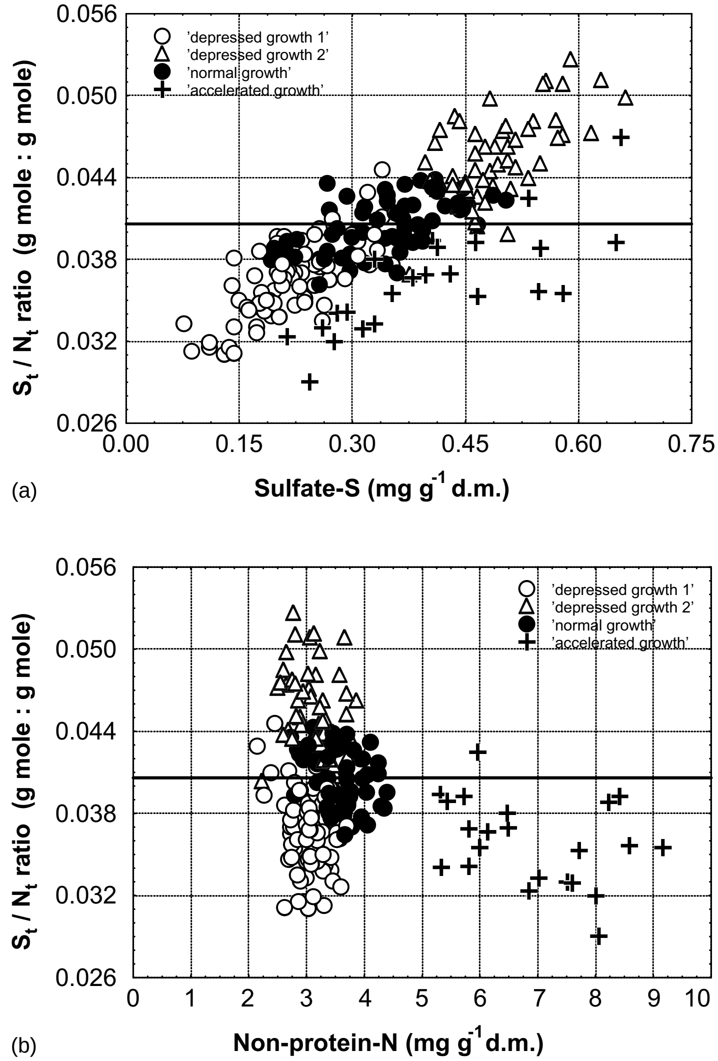

Fig. 2. Graphic display of the four vitality states in the two-dimensional space. The symbols white circles (○) characterize depressed growth 1, white triangles (△) depressed growth 2, black circles (●) normal growth and crosses (+) accelerated growth. (a) S_t:N_t ratio on gram mole basis as function of sulfate sulfur (SO_4^{2-}-S) (b) S_t:N_t ratio on gram mole basis as function of non-protein-N (NPN).

clusters is given by deriving sets of discriminant functions which allow the degree of reparability of the data to be evaluated in terms of these pre-defined clusters and result in a classification scheme to be applied to newly tested needle samples.

In the first step of the discriminant analysis based on the medium Mahalanobis distances, the significance of the four sets of clusters was determined to *F*-tests. The results showed that the previously derived vitality states are significant at the $P < 0.05$ level (details not given). Simultaneously, the diagnostically relevant independent characteristics can be determined because it is not necessary to have all the selected variables determined by NN to characterize the four vitality states. Thus, it is possible to determine the diagnostic optimum number of characteristics P^*, where $P^* < P$, by maximizing the smallest F-ratio between pairs of groups. Here, three discriminant functions were used in the vitality states scheme to separate the data between the respective four clusters,

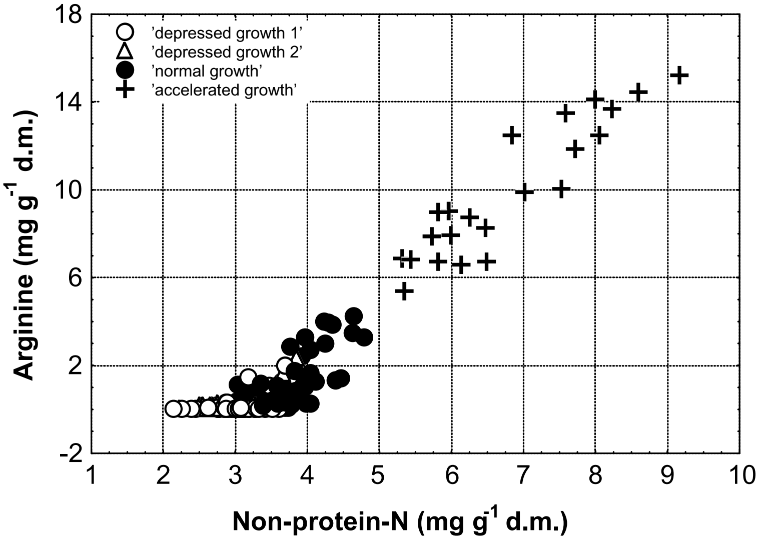

Fig. 3. Correlation between the concentrations of arginine and non-protein-N in 6-month-old Scots pine needles. The symbols white circles (○) characterize depressed growth 1, white triangles (△) depressed growth 2, black circles (●) normal growth and crosses (+) accelerated growth.

and the highest statistical significance was achieved when all variables in the model remained. In Table 5 the coefficients of three functions discriminating between the four vitality states are given in terms of linear combinations of the four biomarkers Arg, NPN, Chl*a*, and SO_4^{2-}-S. Thus, the vitality state can be described by three discriminant functions using the calculated coefficients in Table 5 and the mean values for the needle characteristics in Table 4. The calculation of discriminant functions (F_1, F_2 and F_3) gives the coordinates (discriminant values of $F_1, F_2, \ldots, F_n$) for each vitality state (group centroid) in the discriminant vector space. The dimension of discriminant vector space is determined by eigenvalues, which correspond to discrimination functions, Wilks' λ with the χ^2-test and other statistical quantities. Furthermore, these functions can be applied to the present data set to derive a second classification which can be compared with the classification according to the previously performed cluster analysis. The corresponding results are listed in Table 6. As can be seen from the table, the vitality state 'depressed growth 2' and 'accelerated growth' yield excellent degrees of agreement between both classification schemes (100 and 95.7%, respectively). The highest proportion of different classifications was observed for the vitality states 'depressed growth 1' and 'normal growth', where five samples corresponding to 6.5 or 7% were allocated to other clusters. However, the overall agreement of approximately 95% is quite satisfactory and confirms the previous classification developed with cluster analysis.

Table 5
Coefficients of unstandardized canonical discriminant functions of vitality states after variable reduction

Characteristic	Unstandardized canonical discriminant function coefficients		
	Function 1	Function 2	Function 3
Chlorophyll*a*	0.5818	0.3200	4.5144
Sulfate-S	−0.2332	12.9143	0.6288
Non-protein-N	0.5509	−0.1721	0.7304
Arginine	0.6478	0.0459	−0.2306
Constant	−4.0270	−4.4986	−11.5961

As indicated earlier, the application of the discriminant functions allows the analytical results of new samples to be classified with respect to the vitality states. On the other hand, this procedure is also possible with the aid of Fisher's classification function coefficients, which are simultaneously calculated and are summarized in Table 7. This algorithm enables the assignment of new samples to the vitality states independent of assumed statistical distributions and the training set. Practical application assumes that all theoretically possible functions are included. If this

Table 6
Reclassification of cluster analysis

Prior vitality state (number of cases, cluster analysis)	Predicted vitality state (discriminant analysis)							
	Depressed growth 1		Depressed growth 2		Normal growth		Accelerated growth	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Depressed growth 1 ($n = 77$)	72	93.5	2	2.6	3	3.9	0	0
Depressed growth 2 ($n = 55$)	0	0	55	100	0	0	0	0
Normal growth ($n = 71$)	0	0	5	7.0	66	93.0	0	0
Accelerated growth ($n = 23$)	0	0	0	0	1	4.3	22	95.7

condition is met, comparable results are achieved with Fisher's linear discriminant functions. Another advantages to these classification function coefficients is that less computer equipment is needed. Therefore, Fisher's linear discriminant functions can be used as the mathematical basis of new software solutions for the vitality analysis of Scots pines.

3.5. Applicability of discriminant functions in data analysis

Fisher's linear discriminant functions were used to allocate new needle samples collected from trees in mature Scots pine stands located in Brandenburg at Kahlenberg and Lichterfelde (near Eberswalde), at Kienhorst in the surroundings of Schorfheide and at Wildbahn near Schwedt in 2000 and in Saxony–Anhalt at four sites in Rösa (Nature park Dübener Heath, near Halle–Leipzig–Bitterfeld industrial region) in 2001. Against this background a function value was computed for each vitality state. New samples were assigned to the vitality state for which the corresponding discriminant function had the highest value. The function values were calculated using the needle concentrations analyzed of four selected characteristics (SO_4^{2-}-S, NPN, Arg, Chl*a*) and the calculated coefficients of Fisher's discriminant functions. The resulting classifications were compared with corresponding data from 1992 at the Rösa site and summarized in Table 8.

As can be seen from the table, the three sets of data reveal some interesting temporal variation between the vitality of Scots pines at the Rösa sites in 2001 as well as spatial differences between the vitality of Scots pines in Rösa and at the sites in Kahlenberg, Lichterfelde, Kienhorst, and Wildbahn. The pine stands at the sites Rösa3 and Rösa4 have changed from the vitality state of 'depressed growth 2' to 'depressed growth 1' since 1992, while the pine stand at the Rösa1 sites has changed from the vitality state 'accelerated growth' to 'normal growth'. The pine stand Rösa2 shows no difference between 1992 and 2001. In comparison to the pine stands at Rösa, the Scots pines at Kahlenberg, Lichterfelde, Kienhorst and Wildbahn show spatial differences in tree vitality and mainly exhibit 'depressed growth'. Only the pine stand at Wildbahn displays the vitality state 'accelerated growth', which tallies with the vitality state of the pine stand at Rösa2. These results confirm previously discussed changes in the concentrations of the specific needle characteris-

Table 7
Coefficients of Fisher's linear discriminant functions for assignment of new samples (observations) to one of the grouped vitality states

Characteristic	Classification function coefficients Fisher's linear discriminant functions			
	Function 1	Function 2	Function 3	Function 4
Chlorophyll*a*	41.70950	45.10819	54.02734	56.37436
Sulfate-S	42.89187	88.31047	65.80729	72.02274
Non-protein-N	23.40357	23.23778	25.53402	29.14768
Arginine	−6.04021	−5.89684	−5.80510	−0.87603
Constant	−78.96529	−100.80630	−117.86660	−171.01140

Table 8
Assignment of new needle samples from Rösa (2001) and samples from test sites in Brandenburg (2000), comparison 1992 to assigned samples from 2001 in Rösa

Site	Vitality state		
	1992	2000	2001
Rösa1	Accelerated growth		Normal growth
Rösa2	Accelerated growth		Accelerated growth
Rösa3	Depressed growth 2		Depressed growth 1
Rösa4	Depressed growth 2		Depressed growth 1
Kahlenberg		Depressed growth 1	
Lichterfelde		Depressed growth 1	
Kienhorst		Depressed growth 1	
Wildbahn		Accelerated growth	

tics, which can be attributed to temporal variation of airborne deposition following since the economic changes of 1989 due to the closure of chemical and power plants (Schulz et al., 1999). The pine stands at Rösa and Wildbahn are influenced by sulfur and nitrogen in the form of sulfate as well as ammonia and nitrate. These plant-available compounds have accumulated in the humus layer by atmospheric depositions in recent years and in some cases are now being released on a massive scale by mineralization processes. Initial results of incubation experiments with humus layers (data not shown) confirm the assumption that the Scots pines at the sites Rösa2 and Wildbahn feed mainly on ammonia, while the trees at Rösa3 and Rösa4 mostly take up nitrate. The pine stand at Rösa1 occupies an intermediate state. Therefore, this pine stand changed its vitality state from 'accelerated growth' to 'normal growth', while the pine stands at Rösa2 and Wildbahn show 'accelerated growth', and Rösa3 as well as Rösa4 changed to 'depressed growth' owing to decreasing sulfur depositions. Therefore, the Scots pines are also increasingly using the much greater pool of atmospheric sulfate in the soil solution as a S source and simultaneously reducing their uptake of SO_2 via the needles (Schulz, in preparation). The Scots pines at Kahlenberg, Lichterfelde, and Kienhorst display more or less 'depressed growth' on account of small sulfur and nitrogen deposition. Moreover, this vitality analysis demonstrates how changes in the composition of atmospheric pollutants as well as in mineralization processes of humus layers can be detected by creating complex 'vitality markers' composed of a selection of biochemical, physiological, and nutritional needle characteristics. Thus, computer-aided vitality analysis with 6-month-old pine needles is a useful monitoring tool for the characterization and evaluation of Scots pine vitality states.

4. Conclusion

The results show how the combination of different multivariate approaches can be used to characterize and evaluate vitality states. In particular, cluster analysis and discriminant analysis can be used as differential diagnosis tool to evaluate vitality alterations as a response to complex airborne pollutants and to analyze specific environmental impacts. With Fisher's classification function coefficients calculated, new needle samples can be allocated to the classified vitality states. Therefore, the multivariate results are useful for interpreting and quantifying multiple exposure patterns of airborne pollutants in forest ecosystems. Moreover, the data already collected can provide valuable information for the short-term and long monitoring of atmospheric deposition in forests and its influence on tree vitality.

Acknowledgements

We are grateful to Dr. W. Beck for making his computer facilities at the Federal Research Centre for Forestry and Forest Products, Institute for Forest Ecology and Forest Assessment (Eberswalde) available and for their help during the checking of cross-sectional area increement measurements. We thank I. Geier, M. Herrmann, G. Pelzl and R. Rudloff for their excellent technical support during biochemical analyses, and D. Veit for their help in tree ring analyses. Finally, the authors thank two anonymous reviewers for their helpful comments.

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