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Nitrogen deposition and its impact on forest ecosystems in the Czech Republic - change in soil chemistry and ground vegetation

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A repeated soil survey (1995 and 2006) on 66 ICP Forests pair plots in the Czech Republic revealed a significant relationship between modeled nitrogen deposition and nitrogen concentration in the soil. Nitrogen deposition was modeled for the years 1995, 2004 and 2006. We found a more significant relationship between deposition data in 2004 and soil data in 2006 than between deposition and soil data from the same year 2006. Concentration of total nitrogen in forest soil increased from 1995 to 2006. Forest soil showed effects of increased nitrogen input from the humus layer to around 20 cm depth of mineral soil. The occurrence and cover of nitrophilous species in the herb layer increased from 1995 to 2006 in 25% of the analyzed plots, which corresponds to the nitrogen increase in forest soil. The results suggest that nitrogen deposition still represents a threat for Czech forest ecosystems.

Keywords: Nitrogen Deposition, Soil Chemistry, Ground Vegetation, Ecosystem Changes, Norway Spruce

Introduction

During the whole 20th century, a major impact of human activity on the European environment has been reported, especially on forests. In the Czech Republic, the first reports on the negative effect of sulphur emissions date back to the first half of the 20th century (Singer 1916, Stoklasa 1923). Emissions of sulphur and nitrogen (N) compounds and heavy metals increased continuously and peaked between the mid-70s and mid-80s (Lomský et al. 2002). Effects of SO₂ on forest ecosystems were studied due to its very high levels, and changes in forest soil were mainly investigated (Katzensteiner et al. 1992, Rampazzo & Blum 1992, Wesselink et al. 1995, Lochman et al.

2006, Podrázský & Remeš 2007). After desulphurisation of pollution sources during the 90s, ambient air quality improved rapidly, though forest ecosystems did not respond in the same way. Acid deposition led to base cations leaching from upper soil horizons and then to crown transparency (Materna 1986, Rothe et al. 2002, Lomský & Šrámek 2004). Soil acidification by sulphur and N compounds can cause both a loss of base cations and mobilization of aluminium and other metal compounds, and it has many consequences on forest vitality and vulnerability to other stress factors (De Vries et al. 2000, Schaaf et al. 2004). In recent decades, more attention has been paid to N and its compounds.

De Vries et al. (2014) reported a 25% decrease of N emissions since 1985, but the amount of N emission is still roughly four times as high as in pre-industrial times. Eutrophication continues to be a serious threat to European ecosystems. In 1980 critical loads of nutrient N were exceeded in about 67% of the European area, and are expected to decrease to 42% in 2020 (Posch et al. 2012). Between 1980 and 2020, ecosystems of Central Europe were confirmed to be at high risk of eutrophication (Posch et al. 2012). Critical loads of nutrient N were exceeded in about 100% of the forested area of the Czech Republic in 1994 (Zapletal 2006), and in 95% of the forested area in 2007 (Zapletal 2014).

The effect of N input on the forest ecosystem is complex and disputable – from fertilisation (Solberg et al. 2004, 2009, Jandl et al. 2012) to acidification (Zapletal 1998, 2001, 2006, Augustin et al. 2005, Vícha et al. 2012). Also its potential to cause a loss of diversity in understory vegetation in forest stands is complex (Okland 1995, Wamelink et al. 2008, Kreutzer et al. 2009, Buriánek et al. 2013). The relationship between N addition and biodiversity has been experimentally tested (Reich et al. 2001), showing that N-addition leads to a decrease in biodiversity. Further negative influences of N deposition may be the reduction of root biomass, mycorrhiza and microbial activity, and this in turn may negatively affect nutrient uptake, especially uptake of phosphorus, leading to nutrient imbalance in foliage (Salih & Andersson

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Tab. 1 - Basic characteristics of the plots assessed in both soil surveys (1995 and 2006) and chosen for comparison. The total number of plots was 66.

Parameter	Tree species / range of parameter	Number
Main tree species	Norway spruce	46
	Scots pine	7
	European beech	3
	clear cut between surveys	10
Age in 1995	< 50	0
	51-100	45
	>100	8
	not known	13
Age in 2006	< 50	8
	51-100	28
	>100	25
	not known	5
Altitude	< 400 m a. s. l.	8
	401-800 m a. s. l.	49
	> 800 m a. s. l.	9

1999, Schulze et al. 2005, Mellert et al. 2008, Fujita et al. 2010). In connection with higher N input to the forest stands, a positive fertilization effect on growth is likely. For instance, Austrian forest soil is still deficient in N and stands respond positively to higher N deposition (Jandl et al. 2012). The positive effect of N on forest growth is mentioned by, among others, Solberg et al. (2004, 2009). On the other hand, previous studies confirmed that forest ecosystems turned from N-limited to N-saturated (Mellert et al. 2005, Corre et al. 2007, Kreutzer et al. 2009) and that negative effect of elevated N input causes nutrient imbalance in forest foliage (Braun et al. 2010, Lomský et al. 2011, Lomský et al. 2012).

The aim of our paper was to evaluate changes in soil chemistry and in ground level vegetation composition caused by N deposition between two forest surveys which were done in the Czech Republic.

Material and methods

Ground vegetation assessment

Forest vegetation was monitored within the ICP Forests Programme in accordance with Canullo et al. (2011) at 154 selected plots in the Czech Republic. The assessment was repeated approximately every five years since 1995, taking about three years to assess all the 154 plots. At least three assessments were available for each plot. Basic investigation was carried out during the peak growing season, preferably during the summer, though in certain areas with significant seasonality (e.g., in floodplain forests) spring, or late summer to autumn, were also chosen for survey. The status of the vegetation was evaluated using classic semi-quantitative phytosociological relevés. The assessment was carried out at the center of circular plots with an area of 400 m² (radius of 11.28 m). A modified semi-quantitative seven-item combi-

ned scale of abundance and dominance was used, in accordance with the Braun-Blanquet's concept (Braun-Blanquet 1965), as described in Buriánek et al. (2013). Within ground level vegetation, we focused on species that are considered as indicators of increased N content in the soil and, at the same time, also occur at multiple sites (for example, *Urtica dioica* L., *Geranium robertianum* L., *Impatiens parviflora* DC., *Alliaria petiolata* (M.Bieb.) Cavara & Grande).

Soil survey and data

The first survey was done in 1995-1996 within a national inventory by using a national methodology for sampling (Fabiánek 2004); the second survey was carried out in 2005-2008 within the BioSoil project by using an international methodology (UNECE 2006). The main difference between the two surveys was the range of sampling. Soil samples were taken from soil pit at fixed depth every 10 cm (0-10 cm, 10-20 cm, 20-30 cm, etc.) up to 80 cm in the first survey and at fixed depths of 0-10 cm, 10-20 cm, 20-40 cm and 40-80 cm in the second survey. We assessed selected soil parameters up to 20 cm depth, so that results were comparable as the sampling interval was the same down to 20 cm depth. We chose plots and parameters (pH(CaCl₂), pH(H₂O), total carbon and N concentration, C/N ratio), and soil layers (humus layer, mineral soil 0-10 cm and mineral soil 10-20 cm depth) which were analyzed in both surveys. The number of plot pairs was 66. The main characteristics of the surveyed plots are summarized in Tab. 1.

Chemical analysis was carried out in accordance with the standard operating procedures which are recommended by the ICP Forests programme (UNECE 2006).

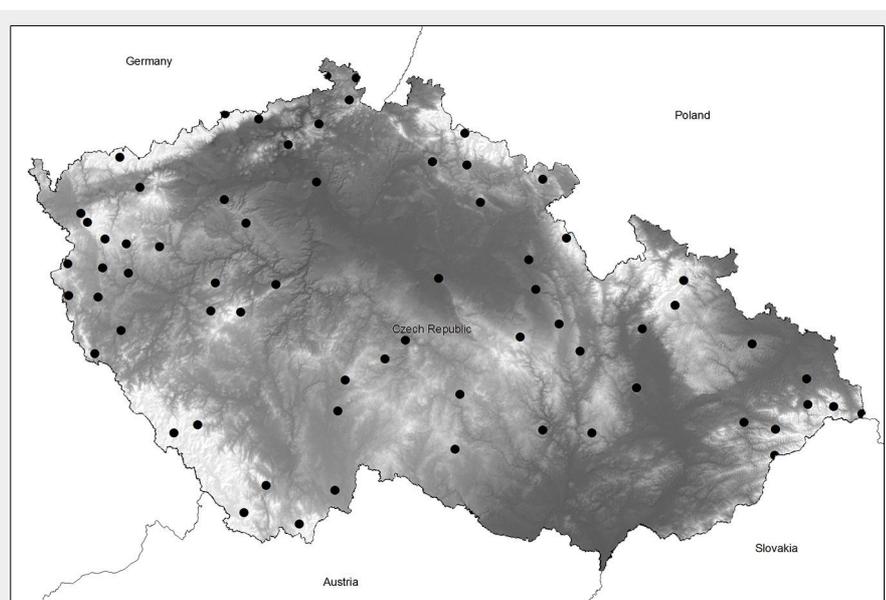
The distribution of plots across the Czech Republic is shown in Fig. 1.

Deposition data

To estimate the atmospheric deposition of N and H⁺ ions at selected plots, we used maps produced by adding wet and dry deposition flux maps with a fine spatial resolution of 1 × 1 km. The method was described in detail by Hůnová et al. (2011) and Hůnová et al. (2014). Wet deposition was calculated based on automated wet-only samples on a weekly basis, analyzed by standard methods with comprehensive QA/QC procedures. Dry deposition was estimated using the inferential method, combining measurements and modeling. A digital map of Czech forests produced from the European digital Land Use map (Corine Land Cover 2000 – <http://etc-lusi.eionet.europa.eu/CLC2000>) was used. All maps were prepared using the software ArcGIS® Geo-statistical Analyst (Johnston et al. 2001). For selected plots total N and total H⁺ ions deposition in 1995, 2004 and 2006 were calculated.

Data analyses

After carrying out the exploratory data

**Fig. 1** – Location of the plots (black dots) selected for the vegetation survey in the Czech Republic.

analysis, their departure from normal distribution was tested. Data sets were tested as dependent or independent samples (according to input data sets); non-parametric tests (Sign test, Wilcoxon pair test, Kruskal-Wallis ANOVA, Median test) were used, because normality was rejected after Shapiro-Wilk W-test. For testing the relationship between deposition and soil parameters, we also used non-parametric test (Spearman R and Kendall tau correlation). Statistical analysis of the data was performed using the package Statistica® Cz version 12.0 (StatSoft Inc., Tulsa, OK, USA).

Results

Vegetation assessment

Overall, the selected nitrophilous indicator species were identified at 74 out of 154 plots (48%) in 2011. At 39 of these sites, their occurrence was rather sporadic (1-2 species with coverage of up to 0.5%). At 13 sites, the occurrence of nitrophilous species was evaluated as significant (usually more species, though with a relatively low coverage of up to 5%). At 22 sites, there was a significant occurrence of nitrophilous species with a high coverage (above 5%). At other sites (80 plots) the occurrence of nitrophilous species was not recorded. At a total of 38 sites (25%), the

Tab. 2 - Non-parametric test of difference in nitrogen concentration and C/N ratio in soil between plots with and without selected nitrophilous species. *P*-values of Kruskal-Wallis ANOVA (KW) and median test (MT) are shown. (w): number of plots with nitrophilous species; (wo): number of plots without nitrophilous species; (FH): humus layer; (M01): mineral soil (0-10 cm); (M12): mineral soil (10-20 cm); (Ntot): total nitrogen concentration in soil; (C/N): carbon to nitrogen ratio in the soil.

Species	w/wo	Test	FH		M01		M12	
			Ntot	C/N	Ntot	C/N	Ntot	C/N
<i>Geranium robertianum</i>	24/121	KW	0.205	0.000	0.074	0.000	0.007	0.000
		MT	0.192	0.000	0.168	0.000	0.023	0.000
<i>Impatiens parviflora</i>	30/115	KW	0.622	0.006	0.571	0.001	0.238	0.001
		MT	0.966	0.045	0.388	0.045	0.203	0.000
<i>Sambucus nigra</i>	33/112	KW	0.320	0.045	0.233	0.001	0.233	0.000
		MT	0.345	0.033	0.152	0.001	0.523	0.000
<i>Urtica dioica</i>	50/95	KW	0.693	0.000	0.011	0.000	0.001	0.002
		MT	0.772	0.000	0.012	0.000	0.000	0.001

presence of nitrophilous species increased during the period analyzed (1995-2011), while a reduction was recorded at seven plots. In addition to the above mentioned species, also *Rubus* spp., *Galium aparine* L., *Lamium purpureum* L. or *Anthriscus sylvestris* (L.) Hoffm. showed an increased occurrence. Regarding woody species, elder (*Sambucus nigra* L.) showed an increased frequency. Also invasive alien species increased, mainly *Impatiens glandulifera* Royle and *Erechtites hieracifolia* (L.) Raf. ex DC.

When comparing groups of sites for the occurrence of selected nitrophilous species, there were no statistically significant differences in N concentration (Tab. 2), while the C/N ratio, which provides a better evidence of ecosystem N saturation, differed among sites (Tab. 2).

Changes in soil

Soil pH increased (Fig. 2) between the two soil surveys, which means that forest soil was less acid in 2006 than in 1995. Con-

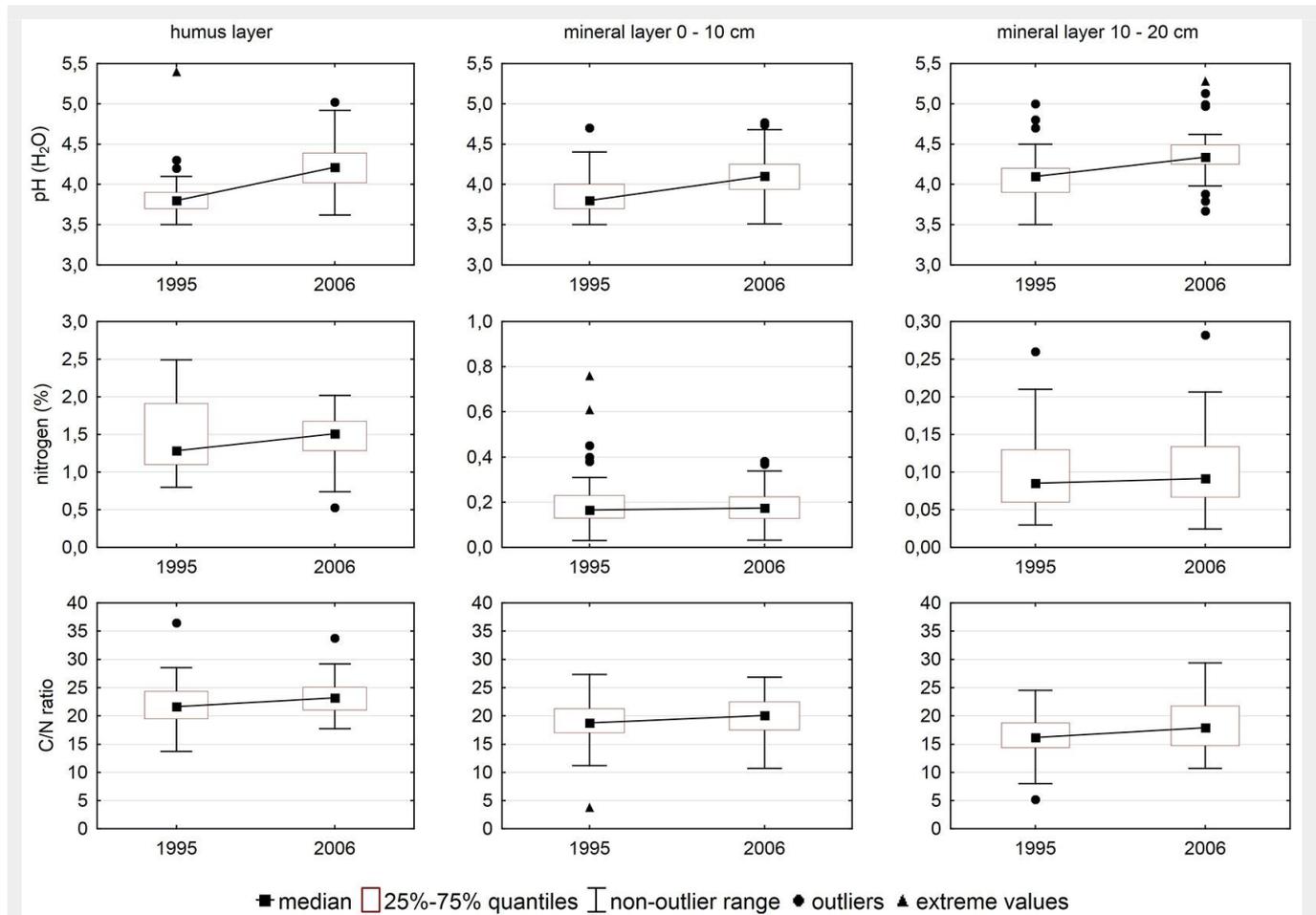


Fig. 2 - Box plots for selected parameters according to soil layer and year of soil survey.

Tab. 3 - Significance of differences in selected soil parameters between 1995 and 2006 after non-parametric pair tests. (ST): Sign test; (WPT): Wilcoxon pair test; (FH): humus layer; (M01): mineral soil (0-10 cm); (M12): mineral soil (10-20 cm).

Parameter	Horizon	n	p-value	
			ST	WPT
pH (CaCl ₂)	FH	65	0.000	0.000
	M01	66	0.010	0.003
	M12	64	0.004	0.000
pH (H ₂ O)	FH	65	0.000	0.000
	M01	66	0.000	0.000
	M12	66	0.000	0.000
carbon	FH	66	0.036	0.006
	M01	66	0.000	0.000
	M12	66	0.000	0.000
nitrogen	FH	66	0.176	0.072
	M01	66	0.000	0.000
	M12	66	0.000	0.000
C/N ratio	FH	66	0.110	0.047
	M01	66	0.005	0.060
	M12	66	0.110	0.015

Tab. 4 - Significance of differences in total nitrogen deposition and total H⁺ ions deposition between 1995, 2004 and 2006 after non-parametric pair tests. (ST): Sign test; (WPT): Wilcoxon pair test; (N): total nitrogen deposition; (H+): total acid deposition.

Pair Comparison	p-value	
	ST	WPT
N 1995 - N 2004	0.000	0.000
N 1995 - N 2006	0.000	0.000
N 2004 - N 2006	0.065	0.121
H+ 1995 - H+ 2004	0.000	0.000
H+ 1995 - H+ 2006	0.000	0.000
H+ 2004 - H+ 2006	0.000	0.000

centrations of carbon and N in soil increased as well. Increase in carbon was higher than increase in N, thus resulting in an increased C/N ratio.

Differences between the two surveys were significant in nearly all cases (Tab. 3).

The difference between total N deposition in 2004 and 2006 was non-significant (Tab. 4), while other differences were highly significant. Deposition was higher in 1995 than 10 years later (Fig. 3).

Relationship between deposition load and soil parameters

Correlations between deposition in 1995 and soil parameters in 1995 and between deposition in 2004 and in 2006 and soil parameters in 2006 were tested. The results are reported in Tab. 5.

In 1995 total N deposition increased with decreasing pH in the humus layer and 0-10 cm mineral soil, while N deposition and N concentration increased in tandem at depth 0-10 cm of mineral soil. In the mineral soil layer at 10-20 cm, total N deposition and N concentration were significantly correlated. When the relationship between total H⁺ ions deposition and soil parameters was tested, there was only a negative correlation with pH in the mineral soil. A stronger effect was visible at 0-10 cm mineral soil depth. No significant correlation was found between total H⁺ ions deposition and N concentration as well as C/N ratio, and between total N deposition and C/N ratio.

In 2006, we tested the influence of total N deposition and total H⁺ ions deposition

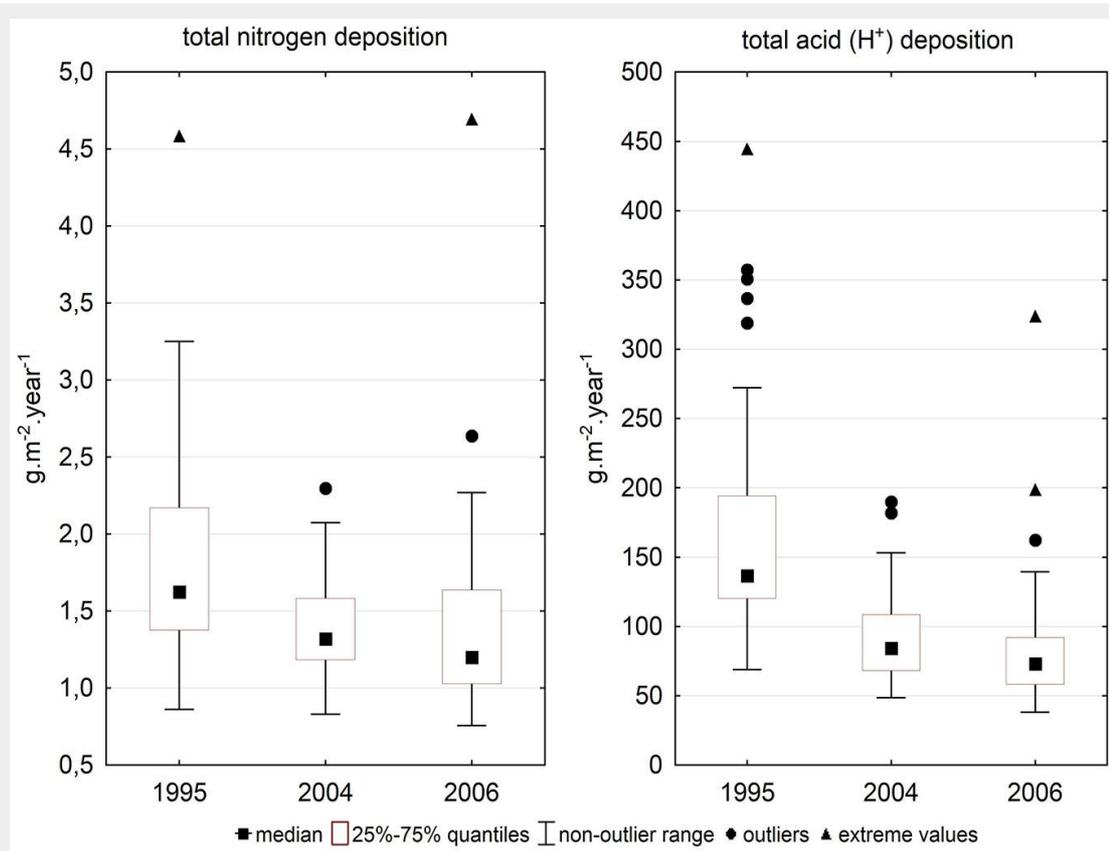


Fig. 3 - Modeled deposition for the selected monitoring plots and years.

Tab. 5 - Correlation between total nitrogen deposition and total H⁺ ions deposition and selected soil parameters. For each parameter/horizon combination, the P-values (first row, in italic) and the correlation coefficient (second row) are reported. (S-R): Spearman R correlation test; (K-T): Kendall Tau correlation test; (FH): humus layer; (M01): mineral soil (0-10 cm); (M12): mineral soil (10-20 cm).

Parameter (1995/2006)	Horizon	Total nitrogen deposition						Total acid deposition					
		1995 (n=66)		2004 (n=66)		2006 (n=66)		1995 (n=65)		2004 (n=66)		2006 (n=66)	
		<i>p-value/</i> corr. coef.											
		S-R	K-T										
pH (CaCl ₂)	FH	<i>0.040</i>	<i>0.030</i>	<i>0.613</i>	<i>0.484</i>	<i>0.294</i>	<i>0.296</i>	<i>0.258</i>	<i>0.238</i>	<i>0.868</i>	<i>0.841</i>	<i>0.113</i>	<i>0.107</i>
		-0.25	-0.18	-0.06	-0.06	0.13	0.09	-0.14	-0.10	0.02	0.02	0.20	0.14
	M01	<i>0.019</i>	<i>0.016</i>	<i>0.835</i>	<i>0.790</i>	<i>0.527</i>	<i>0.648</i>	<i>0.048</i>	<i>0.041</i>	<i>0.721</i>	<i>0.673</i>	<i>0.496</i>	<i>0.483</i>
pH (H ₂ O)	FH	<i>0.013</i>	<i>0.006</i>	<i>0.591</i>	<i>0.548</i>	<i>0.781</i>	<i>0.773</i>	<i>0.109</i>	<i>0.113</i>	<i>0.971</i>	<i>0.991</i>	<i>0.615</i>	<i>0.594</i>
		-0.30	-0.23	-0.07	-0.05	0.03	0.02	-0.20	-0.13	0.00	0.00	0.06	0.04
	M01	<i>0.010</i>	<i>0.007</i>	<i>0.619</i>	<i>0.541</i>	<i>0.592</i>	<i>0.640</i>	<i>0.059</i>	<i>0.041</i>	<i>0.640</i>	<i>0.697</i>	<i>0.590</i>	<i>0.593</i>
Nitrogen	FH	<i>0.350</i>	<i>0.392</i>	<i>0.048</i>	<i>0.042</i>	<i>0.562</i>	<i>0.532</i>	<i>0.265</i>	<i>0.258</i>	<i>0.770</i>	<i>0.778</i>	<i>0.356</i>	<i>0.301</i>
		-0.12	-0.07	0.24	0.17	0.07	0.05	-0.14	-0.10	0.04	0.02	-0.12	-0.09
	M01	<i>0.004</i>	<i>0.005</i>	<i>0.060</i>	<i>0.058</i>	<i>0.141</i>	<i>0.149</i>	<i>0.311</i>	<i>0.345</i>	<i>0.999</i>	<i>0.960</i>	<i>0.681</i>	<i>0.761</i>
C/N ratio	FH	<i>0.309</i>	<i>0.367</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.000</i>	<i>0.993</i>	<i>0.955</i>	<i>0.002</i>	<i>0.002</i>	<i>0.072</i>	<i>0.074</i>
		0.13	0.08	-0.50	-0.35	-0.44	-0.32	0.00	0.00	-0.38	-0.26	-0.22	-0.15
	M01	<i>0.676</i>	<i>0.634</i>	<i>0.035</i>	<i>0.053</i>	<i>0.055</i>	<i>0.055</i>	<i>0.362</i>	<i>0.300</i>	<i>0.023</i>	<i>0.022</i>	<i>0.144</i>	<i>0.146</i>
M12	FH	<i>0.05</i>	<i>0.04</i>	<i>-0.26</i>	<i>-0.16</i>	<i>-0.24</i>	<i>-0.16</i>	<i>0.11</i>	<i>0.09</i>	<i>-0.28</i>	<i>-0.19</i>	<i>-0.18</i>	<i>-0.12</i>
		<i>0.809</i>	<i>0.710</i>	<i>0.593</i>	<i>0.559</i>	<i>0.225</i>	<i>0.197</i>	<i>0.578</i>	<i>0.610</i>	<i>0.373</i>	<i>0.367</i>	<i>0.344</i>	<i>0.350</i>
	M01	0.03	0.03	-0.07	-0.04	-0.15	-0.11	0.07	0.04	-0.11	-0.08	-0.12	-0.08

on soil chemistry for the same year of the soil survey (in 2006) and we also tested the influence of deposition calculated in 2004 and its correlation with soil chemistry in 2006. We found more significant results for deposition data in 2004 and soil data in 2006 than for deposition data in 2006 and soil data in 2006. Total N deposition in 2004 increased with increasing N concentration in the humus layer and in the mineral soil at 10-20 cm depth in 2006. Total N deposition in 2004 decreased with increasing C/N ratio in the humus layer and mineral top soil at 0-10 cm in 2006. Correlation between total N deposition in 2006 and selected soil parameters in 2006 was significant only for C/N ratio in the humus layer. Total H⁺ ions deposition in 2004 was negatively correlated with C/N ratio in the humus layer and mineral top soil at 0-10 cm in 2006. Total H⁺ ions deposition in 2006 was significant only for C/N ratio in the humus layer in 2006.

Discussion

During the past 20 years, a higher proportion of nitrophilous species was detected in Czech forests (Buriánek et al. 2013), likely due to the elevated N deposition. Similar results have been reported across Europe (Gilliam 2006, Fischer et al. 2012). The gradual replacement of oligotrophic species by eutrophic species as a response to N deposition has been observed at the European scale based on long-term monitoring at 28 forest sites with a total of 1335 permanent forest floor vegetation plots (Dirnböck et

al. 2014). The spread of nitrophilous species throughout the forest suggests the N saturation of forest ecosystems. While the overall N deposition slightly decreased, in some regions there was again a weak increase (Hůnová et al. 2014). A slight but significant increase of nitrophilous species within the ICP Forests monitoring plots in Europe in connection with N deposition is reported also by Seidling et al. (2008).

Changes in total N concentration in forest soil between the surveys in 1995 and in 2006 and correlation between total N deposition and total N concentration in soil were significant. The relationship between 2004 deposition and 2006 soil chemistry was more significant than the relationship between deposition load and soil chemistry in the same year. This confirms that there is a time lag between the input of the compounds to the forest and their measurable effects on the ecosystem. Results from N deposition measurements across Europe confirm our findings – there is a slight decrease in N deposition, but critical deposition loads for nutrient N are exceeded in more than half of the monitoring plots in Europe (Seidling et al. 2014). Critical loads for inorganic N deposition were exceeded on about a third to a half of the monitored forest plots of the ICP Forests Level II network across Europe (Waldner et al. 2015). Elevated inorganic N concentrations in soil solution occurred more frequently in these plots (Waldner et al. 2015). Indications of nutrient imbalances, such as low magnesium concentration in foliage or

discoloration of needles and leaves, were rare but appeared more frequently on plots where the critical limits for soil solution were exceeded (Waldner et al. 2015). Also in the Czech Republic there are regions where elevated N concentration in soil leads to nutrient imbalance, especially between N and phosphorus and N and magnesium (Lomský et al. 2011, 2012).

Our results show that all evaluated soil horizons (humus layer and mineral soil up to 20 cm depth) are affected by high N input. This is an important confirmation that not only the soil surface is influenced. It clearly shows that forest soil is already saturated by N in some regions and that there is mid- or long-term influence of N compounds on forest soil. If the soil is N-saturated, there is a risk of high leaching of N compounds to the ground water. In the Czech Republic, the monitoring of runoff water in small forest catchments confirms this finding, as the concentration of nitrate is quite high (> 5 mg l⁻¹) and related with high N deposition (Vícha et al. 2012).

Moreover, the realistic nitrogen deposition in Czech forests is likely to be much higher than the modeled values used for our analysis. The N deposition is likely to be substantially underestimated not accounting for several important non-measured compounds, such as NH₃ and HNO₃ (g), and contribution of occult deposition, as recently shown by Hůnová et al. (2016).

Conclusions

There is a significant influence of N depo-

sition on forest soil in the Czech Republic; between 1995 and 2006, concentration of total N increased in the upper 20 cm of soil. Deposition load exceeded the critical threshold for forest ecosystem in the Czech Republic. The results suggest that N deposition still represents a threat for forest ecosystems in the Czech Republic.

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