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Ground vegetation as an important factor in the biodiversity of forest ecosystems and its evaluation in regard to nitrogen deposition

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ABSTRACT: We documented the current typological and phytosociological characterisation of the ground vegetation as an essential component of biodiversity in 154 Czech forest monitoring plots and to describe its changes during the past 15 years in regard to the deposition and concentration of nitrogen in the soil. Plots were classified as vegetation units in accordance with the UNECE and FAO nomenclature and on the basis of their potential natural vegetation and compared in terms of the occurrence and coverage of the indicative selected nitrophilous species. In all the soil horizons tested statistically significant differences in the C/N ratio were observed between areas with and without the presence of certain selected nitrophilous species (*Geranium robertianum, Impatiens parviflora, Sambucus nigra, Urtica dioica*). In the areas with the presence of the *Geranium robertianum* and *Urtica dioica* species, statistically significantly higher concentrations of nitrogen were recorded in some soil horizons than in those areas with the absence of these species. The findings concerning the influence of nitrogen on nitrophilous herbaceous indicators were compared with the European results obtained in the framework of the ICP Forests international programme and with those of other foreign studies.

Keywords: forest monitoring; nutrificiation; nitrophilous species; vegetation diversity

Biodiversity is nowadays considered to be one of the principle criteria for the sustainability of forest production and for ensuring its non-production functions. Within the framework of international cooperation, in which the Czech Republic is also involved, indicators of biodiversity in forest ecosystems and improved and refined methods of its measuring and evaluation were developed. Quite an extensive set of objective and subjective indicators are available on the basis of constant monitoring and regular evaluation in countries with a developed forestry industry. During recent years, biodiversity and its assessment have occupied an important position in the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests), in which the Forestry and Game Management Research Institute has been actively involved.

The main component of forest ecosystems, in addition to tree edificators, is the ground vegetation. The individual vegetation layers comprise an important feature of the overall biodiversity of forest ecosystems. Their composition, diversity and structure constitute both important factors in the assessment of their biodiversity and also important bioindicators of environmental changes. Vegetation is a source of the primary production of ecosystem; it controls gas exchange with the atmosphere and plays an important role in the biocycles of both water and nutrients within the ecosystem. It is also an important indicator of the status of forest ecosystems for the relatively easy and inexpensive assessment of ground vegetation monitoring and constitutes an acknowledged basis for biodiversity assessment (Buriánek 1993; Neumann, Starlinger 2001; Petriccione 2002; Seidling 2005; Salemaa, Hamberg 2007). Knowledge of the autecology of many different species enables the interpretation of changes in vegetation in relation to corresponding changes in environmental

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factors. Vegetation, in its entirety, as well as the individual species as indicators are equally considered to be specific subjects for studying the levels of the critical loads of ecosystems.

The objectives of the vegetation assessment are to capture and to document both the current typological and phytosociological characteristics of the vegetation and their variations at individual sites and also any changes that are taking place in time based on the influence of natural and anthropogenic factors. Long-term study of vegetation in areas of intensive monitoring is intended to provide data for the overall study of changes in forest ecosystems in relation, for example, to the soil conditions, micro- and mesoclimate, etc.

It is generally known that vegetation cover responds to environmental changes with great sensitivity and with appropriate speed. Numerous domestic and foreign studies concerning the relationship between the forest vegetation and the environment show that any change in abiotic factors can lead to considerable changes in the vegetation (e.g. Ambros 1990). One of the global problems most ecosystems have been facing during recent decades is the exceeding of an acceptable level of nitrogen. With the development of industry, the manufacture of agricultural fertilisers and the growth of the urban population, nitrogen has frequently become a superfluous nutrient and, for some natural systems, even a harmful substance. An excess of nitrogen is suitable for those plants that are able to use it effectively (e.g. *Urtica dioica, Chelidonium majus, Impatiens, Sambucus nigra, Fraxinus excelsior*), while it eliminates plants and organisms that are able to cope with its lack (orchids, cranberries, lichens) (Hofmeister et al. 2002). In the forests of Central Europe even in the mid-20th century the raking of stall bedding was practiced, in addition to grass mowing, also, to a limited extent, forest grazing (goats) and coppicing, which prevented the accumulation of organic matter and thereby the accumulation of nitrogen, and which created suitable living conditions for rare and endangered species of plants and animals (Hofmeister et al. 2009).

Nitrogen is an essential nutrient and it is also one of the most important macrobiogenic elements. Unlike other nutrients, however, it is rarely contained in rocks, and plants therefore obtain it almost exclusively in the form of nitrates or of ammonium ions from the decomposition of organic matter, from atmospheric deposition or through microbial fixation that reaches the soil solution. Important for evaluation of a potential source of nitrogen is the total amount (the reserves) in the forest floor; important for the characterisation of its accessibility is its C/N ratio (Binkey 1986; Fisher, Binkley 2000). Eutrophication, together with acidification, pertains to one of the most significantly negative processes in natural ecosystems. It represents the excessive supply and accumulation of nitrogen and phosphorus compounds in an ecosystem, as a result of which a change in the balance of nutrients in the soil occurs (Nilsson, Cowling 1992; Crowley et al. 2012). This manifests itself negatively in regard to the intake of other essential elements such as magnesium, in the loss of a large number of soil microorganisms and fungi and also of many sensitive plant species (Diekmann, Dupré 1997). The critical load concept originated in the 1980's and was based on the framework of the Geneva Convention, signed in 1979 (Johannessen 2009).

A critical load is defined as the maximum permissible dose of pollutants that does not yet cause any chemical changes that could lead to long-term damage to the most sensitive components of ecosystems (Nilsson, Grennfelt 1988). The critical load for nutrient nitrogen was also defined as the highest possible level of nitrogen deposition, in the form of oxidation and reduction compounds, that does not act detrimentally either on the structure or on the functioning of the specific type of ecosystem (UBA 2004). Critical loads for nutrient nitrogen are of dual nature – critical loads of nutrient nitrogen calculated on the basis of simple mass balance and empirical critical loads of nitrogen based on observation and scientific experiments. Critical loads facilitate estimating the quantity of pollutants in the air that needs to be reduced in order to avoid long-term damage to natural ecosystems. In recent years, critical loads of nitrogen have been simulated and updated for different types of societies (van Dobben et al. 2006; Bobbink et al. 2011).

Despite the implementation of extensive technological measures to reduce emissions, the quantity of the forms of nitrogen that are emitted has not changed significantly. In addition to the possibility of the amount of nitrogen increasing from atmospheric deposition, there is also a risk of an increase in the mobility of soil nitrogen. Some authors discussed the ecosystem nitrogen saturation in relation to an excess of nitrogen (Aber et al. 1989; Gundersen 1991; Gundersen et al. 1998) that proceeds in three stages, which are initially manifested as soil acidification, imbalances in nutrition, the reduction of growth processes and an increased leaching of nitrogen, additionally as changes in the vegetation species composition. The ecosystem nitrogen saturation is also reflected in a reduction of the C/N ratio in soils, which is considered to be one of the significant indicators of saturation.

The evaluation of acidification and of the eutrophication of forest ecosystems has, to date, relied on calculations of the critical loads of sulphur and of nitrogen and these loads being exceeded by atmospheric depositions (Skořepová et al. 2007, 2009, 2010). In addition to atmospheric deposition, decaying organic matter may also be another significant source of nitrogen. So far, however, calculations of the critical loads of nitrogen have not yet been considering this additional source of nitrogen in the ecosystem. The current status of the calculations has been reflecting only one limit value of nitrogen concentration in the soil solution in respect of a specific type of vegetation, i.e. its critical concentration (de Vries et al. 2007). The concentration of nitrogen in the soil solution has a decisive impact not only on the rate of leaching of N from the ecosystem, but also determines the vitality of the specific plant society. It also depends on the level of nitrogen concentration in the soil solution whether an excess of nitrogen will cause eutrophication, followed by a change in the composition of the vegetation or whether the ecosystem will suffer from a lack of nitrogen, or it will be in a stable condition and adequately supplied with nitrogen.

Apart from eutrophication an excess of nitrogen has another negative consequence. The rate of tree growth increases in association with the higher temperature (Fischer et al. 2007). Trees in mountain forests receive, on the one hand, an excess of nitrogen and, on the other hand a lack of the basic cations that have been washed out of the soil by acid rain. With faster growth, as has been demonstrated in Europe since the 1950's (Spiecker 1999), disproportions of nutrition are created. The fast growing spruce wood matures improperly, the length increments are enormously large and the trees become susceptible to mechanical damage – they break very easily in strong winds, when exposed to ice or to wet snow. The increased quantity of nitrogen in the tree tissues is also very attractive to pathogenic pests (Throop, Lerdau 2004; Meyer et al. 2008),

METHODS

The monitoring of forest vegetation in the Czech Republic is carried out in the framework of the ICP Forests Programme and in accordance with the most recent updated version of Chapter VII-1 Evaluation of ground vegetation in the Manual on methods and criteria for harmonised sampling, assessment, monitoring and analysis of the effects of air pollution on forests (Canullo et al. 2011), first published in 1997 and edited and supplemented during the following years. In total 146 Level 1 plots and 8 Level 2 plots pertaining to the ICP Forests international network were assessed and processed.

Defined as the ground vegetation is any plant component of the ecosystem, with the exception of the tree layer and the epiphytic and epilithic mosses and lichens, i.e. the shrub layer ‑ E2, (woody plants, such as shrubs, small trees and climbers from 0.5 to 5 m in height), the herbal layer ‑ E1 (herbs and tree seedlings of up to 0.5 m in height and dwarf shrub species such as the genus *Vaccinium*) and the moss layer ‑ E0 (terrestrial bryophytes and lichens). Assessments have been carried out at five-year intervals since 1993, systematically since 1995, meaning that at least three assessments have already been conducted at all of the sites.

Basic investigations are carried out during the peak growing season, preferably during the summer aspect, and in case of significantly different seasonal aspects (e.g. in floodplain forests) the spring or the late summer assessment has also been realized. A list of all the species, including tree seedlings, was recorded in all three layers. The status of the vegetation was evaluated using classical semi-quantitative phytosociological relevés. Assessment was carried out on circular plots, located at its centre, with an area of 400 m^2 (a radius of 11.28 m).

Barkman's modification (Barkman et al. 1964) of semi-quantitative Braun-Blanquet's seven-item combined scale of abundance and dominance (Braun-Blanquet 1965) was used:

- r – very rare species, mostly only one or a few individuals with negligible coverage,
- + – rare species (at least two individuals on the plot) or occasionally occurring, but with low coverage,
- 1 – frequently occurring species, but with low coverage, or less frequently occurring species with higher coverage, but not greater than 5% (these are often sporadic shrubs or rarer grasses),
- 2a – very common (abundant) species, with a high number of small individuals and with coverage of about 5%, or a lower number of larger plants with 5–12.5% coverage,
- 2b – similar to 2a, however the coverage always represents 12.5–25% of the total area,
- 3 – species with 25–50% coverage,
- 4 – species with 50–75% coverage,
- 5 – species with 75–100% coverage.

In the case of the r, +, 1 and 2a categories what is primarily considered is abundance, while in the higher category levels it is only the coverage of the individual species. At the same time, the total coverage of the ground vegetation was determined and also the coverage of the individual layers to the nearest 5% and the canopy closure was also estimated. The nomenclature follows Flora Europaea (Tutin et al. 1968–1980, 1993) for vascular plants. In regard to the species that are not listed in this publication and in those cases in which a more accurate taxonomic determination is required the Key to the Flora of the Czech Republic (Kubát et al. 2002) was used. The nomenclature for bryophytes follows Frey et al. (1995), for lichens Wirth (1995).

Both typological (Průša 2001) and phytosociological characteristics of the vegetation (Moravec et al. 1995; Chytrý et al. 2010) were implemented at all monitored plots. The classification of forest types in accordance with the nomenclature of the Report on the State of Europe's Forests (Forests Europe, UNECE and FAO 2011) was used for the inclusion of individual plots into specific vegetation units. The basis of this classification is simply the species composition of the tree layer, regardless of whether these woody plants correspond with the natural species composition or whether they constitute man-made monocultures of coniferous trees. For comparison, the individual plots were also categorised to nine vegetation units on the basis of their potential natural vegetation, according to the Catalogue of Habitats of the Czech Republic (Chytrý et al. 2010), using the work of Neuhäuslová et al. (1998). The same system was used in the publication on the monitoring of the forest condition (Fabiánek et al. 2004). This classification provides a clearer view on the character and diversity of habitats, since it is based on the characteristics of ground vegetation that is usually at least partially maintained, even in those stands in which the natural species composition has been totally changed.

Subsequently, based on bibliographical research (Sýkora 1959; Diekmann, Dupré 1997; Krpeš 2005) and utilising Ellenberg's indicator values (Ellenberg et al. 2001), a list of the suitable phytoindicators of a possible nitrogen load was prepared and also the dependence of the presence and the coverage of selected indicator species on both the nitrogen deposition and content and on the C/N ratios in the humus layer and in the upper mineral soil layer was determined. Chemical analysis of the soil samples to determine the concentration of nitrogen was carried out by the Test Laboratory of the Forestry and Game Management Research Institute in accordance with the standard operating procedures, using the CNS analyser for total nitrogen. Methods are described in ICP Forests manual, part X – Soil sampling and analysis (ICP 2010).

Statistical evaluation of the data obtained was conducted using the STATISTICA 10 programme (SPSS, Tulsa, USA). After carrying out the exploratory data analysis the normality was tested (using the Shapiro-Wilk *W*-test) and, based on the results of this testing, appropriate parametric and non-parametric tests were selected (i.e. the sign test, Wilcoxon pair test, Kruskal-Wallis ANOVA and median tests) to identify the relationships between selected parameters.

RESULTS

The assessment of biodiversity was based on the monitoring of ground vegetation at 154 sites pertaining to ICP Forests. In almost every case these represent normal commercial forests, of which only a small proportion can be considered as near-natural forest communities. Most of the sites were greatly affected and altered by long-term forest management. The determining factors consisted of particular changes in the specific composition of the tree species (the transformation to spruce or pine monocultures), which, of course, was reflected in changes in the ground vegetation. It differs more or less from the natural status at most of the plots. Species impoverishment occurs frequently, including important indicator species. As a result of high stocking, especially in the younger age classes, the ground vegetation has remained only as a sporadic residue in some coniferous monocultures. Elsewhere, on the other hand, the onset of a number of invasive species is apparent, often those of a synanthropic and ruderal character. At some sites the ground vegetation is ecologically undefined, without any significant indicator species. In the course of the classification of vegetation units, in many cases of transitional types it was necessary to assess which of the indicators are predominant and decisive. Therefore, due to these circumstances, a more detailed phytosociological and typological classification of certain sites is difficult and sometimes even problematic.

When classifying the forest types following the nomenclature of the Report on the State of European Forests (Forests Europe, UNECE, FAO 2011) the monitored sites were classified in nine units.

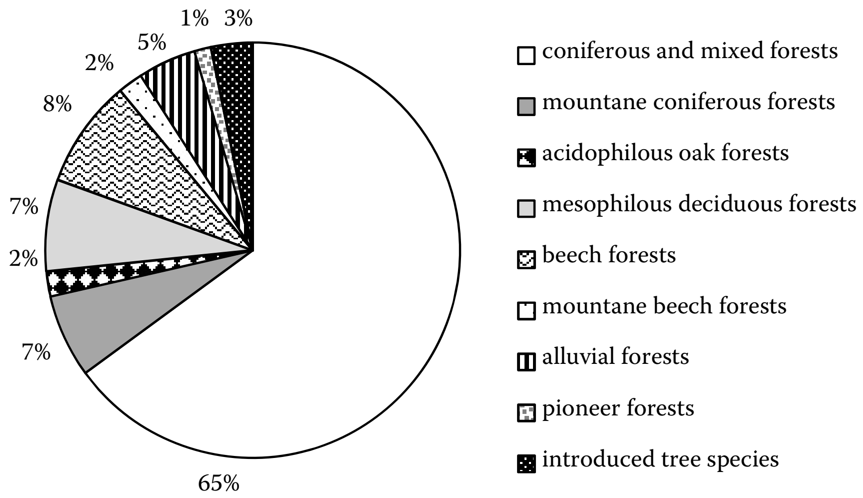

Fig. 1 Vegetation types according to UNECE and FAO nomenclature on monitoring plots

By far the most widely represented is the category of coniferous and mixed forests (65%); the other units reach a maximum of 8% (Fig. 1). In regard to the presence of vegetation units on the basis of their potential natural vegetation (Fig. 2), the most widely represented are acidophilous beech forests, followed by herb-rich beech forests, acidophilous oak forests, oak-hornbeam forests and pine-oak forests. Mountain and waterlogged spruce forests are represented to a lesser extent. Other units were found only at a few sites. Thermophilous oak forests and ravine forests are the richest in plant species, herb-rich beech forests, alluvial forests and oak and hornbeam-oak forests are also above average, while in the case of these last two units other species also occur in the spring aspect. The acidophilous oak and beech forests are significantly poorer in species; the smallest number of species can be anticipated in pine-oak forests.

The presence of these vegetation units at these sites roughly corresponds with their overall presence in the forest societies in the Czech Republic (Fig. 3), which has been calculated based on the area of the corresponding sets of forest types and prepared on the basis of data from a long-term detailed typology survey of forests in the CR (Plíva, Žlábek 1986). According to these data, in the Czech Republic there is a higher proportion of forest type groups corresponding to herb-rich beech forests, to the detriment of acidophilous beech forests. This difference is explained by the impoverishment of the richer habitats of herb-rich beech forests due to forest management and especially due to changes in the species composition that are manifested by changes in vegetation. Another difference is the lower proportion of forest type groups corresponding to pine-oak forests than on the assessed plots, which is probably due to the

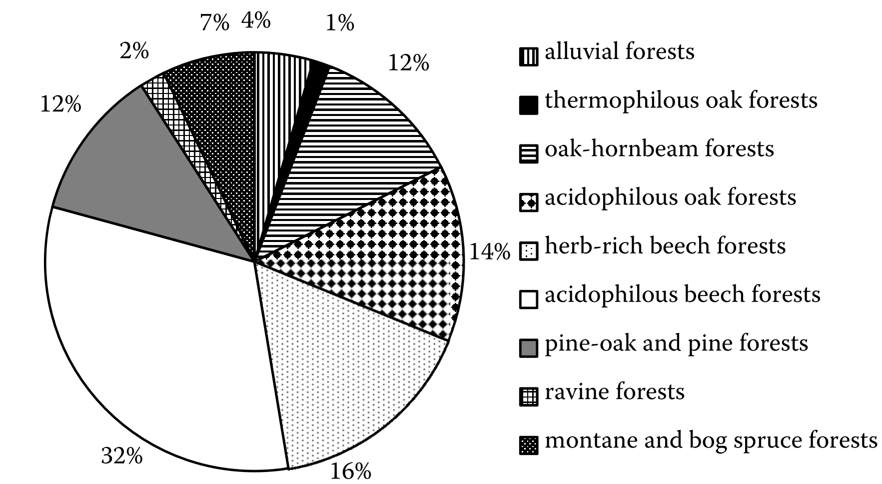

Fig. 2 Vegetation units according to potential natural vegetation on monitoring plots

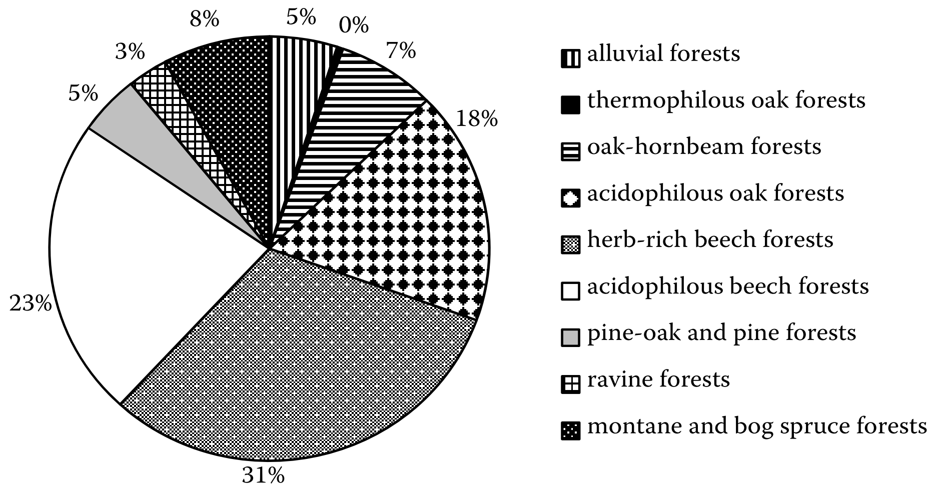

Fig. 3 Vegetation units according to Czech national forest typology survey

fact that, in some cases, these forest type groups were included in the related unit of acidophilous oak forests.

Alluvial forests of the *Alnion incanae* alliance occur at seven sites, in habitats on alluvial and gley soils that are at least episodically flooded and that are also significantly affected by groundwater that occasionally appears above the soil surface. Represented are alluvial-riparian forests of the 1st forest altitudinal vegetation zone (FAVZ), i.e. elm-oak forests (*Querco-Ulmetum*), bird cherry-ash forests (*Pruno-Fraxinetum*) and elm-ash forests (*Fraxino pannonicae-Ulmetum*), in complex with poplar-ash forests (*Fraxino-Populetum*) and also alluvial forests with black alder and grey alder along the smaller water streams and springs (the *Alnenion glutinosoincanae* suballiance). One site in the Bohemian-Moravian Highlands corresponds to spruce-alder forests (*Picea abietis-Alnetum glutinosae*).

Thermophilous oak forests, primarily of the *Quercion pubescenti-petrae* alliance, characterised by thermophilous and xerophilous Submediterranean-Pontic species in the herb layer are represented only at two sites of the 1st and 2nd FAVZ. Oak forests with *Potentilla alba* (*Potentillo albae-Quercetum*) and with *Lathyrus versicolor* (*Lathyro versicoloris-Quercetum pubescentis*) are represented.

Oak-hornbeam forests of the *Carpinion* alliance constitute an abundantly represented vegetation type in the oak forest zone. Most frequently found in the area of the Bohemian Massif are oak-hornbeam forests with *Melampyrum nemorosum* (*Melampyro nemorosi-Carpinetum betuli*) while in the Carpathian area there are oak-hornbeam forests with hairy sedge (*Carici pilosae-Carpinetum*) and in the Panonnian area oak forests with spring primrose (*Primula veris-Carpinetum betuli*). Oak forests (*Stellaria holosteae-Tilietum cordatae*) have been identified at plots in the central Elbe region. These represent a poorer type of mesophilic oak forests with an admixture of linden and a thin occurrence of hornbeam. Linden-oak-hornbeam forests (*Tilio cordatae-Carpinetum betuli*) with a natural admixture of spruce have also been identified at several sites in Silesia. These are characterised by the loss of thermophilic elements and the higher proportion of hydrophilic species of alluvial forests. At several sites there has already been a transient drift towards beech forests. At some sites the autochthonous tree layer has been replaced by spruce, pine or larch. Their original herb layer, however, has also been partially retained.

Acidophilous oak forests of the *Genisto germanicae-Quercion* alliance are a particularly abundant vegetation type on the nutrient-poor substrates in the oak forest zone, particularly in Bohemia. They are represented mostly by woodrush oak forests (*Luzulo albidae-Quercetum petraeae*) and in damper localities by *Molinia* oak forests (*Molinio arundinaceae-Quercetum*). The natural tree species composition, dominated by oak, has been preserved at a few sites only. Oak has often the scarce presence or is restricted to a shrub layer or is completely missing. At higher altitudes a slight admixture of beech occurs. Some plots indicate a transition to pine-oak forests, with a probably higher natural occurrence of pine, which, however, was also favoured by the intensive forest management creating clear-cut areas where pine as a pioneer tree could play an important role. In addition to pine plantation, conversions to spruce monocultures are also frequent, sometimes in mixtures with larch. At two plots cultures of the exotic species – white pine and Douglas fir have been planted.

Herb-rich beech forests of the *Fagion sylvaticae* alliance are found from the 3rd to the 6th FAVZ in several different variants rich in herb species. The previous much larger admixture of fir has remained at some plots. Bittercress beech forests (*Dentario enneaphylli-Fagetum*) are definitely the most abundant community. Especially in Moravia wood melick beech forests (*Melico uniflorae-Fagetum sylvaticae*) also appear, as hairy sedge beech forests (*Carici pilosae-Fagetum*). Viola beech forests (*Viola reichenbachianae-Fagetum sylvaticae*), characterised by the absence of the genus *Dentaria*, are limited to the areas of the Krušné hory Mts. and Doupovské hory Mts. At many plots in this category the vegetation is of a transient character; in the lower vegetation zones towards hornbeam-oak forests, at higher zones towards acidophilous beech forests or beech-spruce forests. Herb-rich beech forests also represent significantly anthropogenically influenced communities. There are frequent conversions to spruce monocultures, and the impact of these interventions (impoverishment and secondary acidification) on the original structure of the herb layer is significant. At some sites herbaceous and moss vegetation is almost absent (i.e. *Fagetum nudum* – naked beech forests).

Acidophilous beech forests of the *Luzulo-Fagion sylvaticae* alliance are the most frequently represented unit in the mineral-poor soils of the beech zone, while fir was preserved at certain sites only residually. In most cases they are represented by woodrush beech forests (*Luzulo luzuloidis-Fagetum sylvaticae*) and in some places by small-reed grass beech forests (*Calamagrostio arundinaceae-Fagetum sylvaticae*). In the 6th FAVZ in the Šumava Mts., Krkonoše Mts. and in the Orlické Mts. spruce-beech forests occur (*Calamagrostio villosae-Fagetum sylvaticae*), with a natural proportion of spruce and the occurrence of some mountain species. Mostly alternating in the dominant role are *Deschampsia flexuosa* or *Vaccinium myrtillus*, and rarely *Calamagrostis arundinacea*, *Oxalis acetosella* or *Poa nemoralis*. The main diagnostic species is *Luzula luzuloides*. The species composition of the herb layer is very monotonous. The natural forest stands are frequently replaced by spruce monocultures, sometimes with a proportion of larch.

Pine-oak and pine forests of the *Genisto germanicae-Quercion* and *Dicrano-Pinion sylvestris* alliances occur azonally on nutrient-poor and acidic substrates, predominantly in Western Bohemia in the area between Pilsen and Tachov, in the Česká Lípa district and in Southern Bohemia. They are represented by cranberry pine-oak forests (*Vaccinio vitis-idaeae-Quercetum*). Two sites in the Elbe region and one in South Moravia are classified as fescue pine-oak forests (*Festuco ovinae-Quercetum roboris*). They are characterised by pedunculate oak and the absence or only sporadic occurrence of the genus *Vaccinium* and the predominance of sheep fescue (*Festuca ovina*). In both these types, oak was suppressed by management to such a degree that nowadays almost pure pine monocultures are dominant. Oak has remained mostly as a shrub layer. The vegetation at one site in the North-Bohemian sandstone plateau can already be considered as transitional to bog bilberry pine forests (*Vaccinio uliginosi-Pinetum sylvestris*). They are characterised by a boggy mineral soil, the absence of oak, a possible admixture of autochthonous spruce and a moss layer with high coverage.

The **ravine forests** of the *Tilio-Acerion* alliance are associated with small-scale forest communities on detritus and boulder weathering residues with azonal occurrence. They are characterised by the high presence of scree wood species (linden, maple, ash and elm). These were recorded at three sites, although not in all these cases are they developed in their pure form. They occur in the zone of beech forests, usually herb-rich beech forests. Their associations are present: maple forests with perennial honesty (*Lunario redivivae-Aceretum*) and ash forests with perennial mercury (*Mercuriali perennis-Fraxinetum excelsioris*). Characteristic is the high coverage of ferns. Due to their difficult accessibility because of the relief (steep, rocky slopes) and their usual inclusion in the category of protective forests, these communities are well preserved in a near nature status.

The mountain and waterlogged spruce forests of the *Piceion abietis* alliance are represented at eleven sites in the 7th to 8th FAVZ, although the natural occurrence of spruce somewhere else is also documented. At most plots there are small-reed grass spruce forests (*Calamagrostio villosae-Piceetum abietis*) or spruce forests with broad buckler fern (*Dryopterido dilatatae-Piceetum abietis*). Two plots in the Krušné hory Mountains have been classified as permanently waterlogged spruce forests (*Sphagno-Piceetum abietis*).

In order to evaluate the relationship between the vegetation and the content of nitrogen the following species of nitrophilous forest plants were selected, indicating a soil rich in organic nitrogen compounds: *Aegopodium podagraria*, *Alliaria of-*

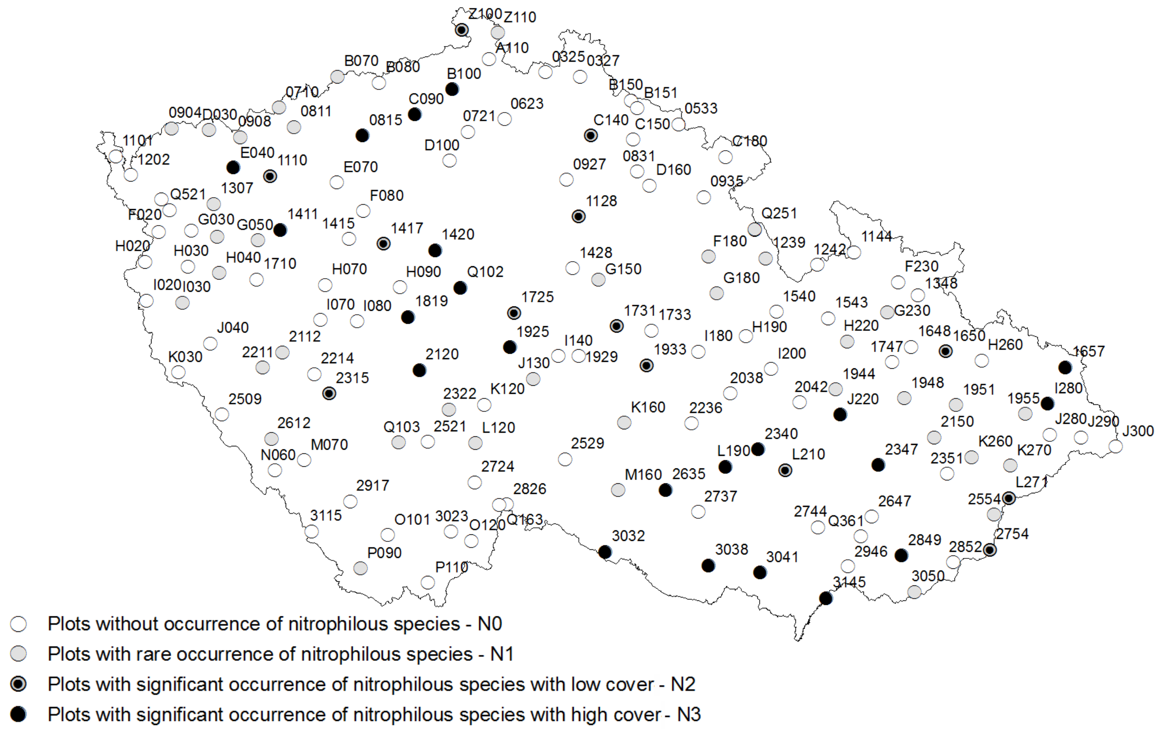

Fig. 4 Distribution of plots according to the occurrence of nitrophilous species

ficinalis, *Anthriscus sylvestris*, *Arctium lappa*, *Calystegia sepium*, *Chelidonium majus*, *Chenopodium album*, *Galium aparine*, *Geranium robertinanum*, *Impatiens parviflora*, *Lamium purpureum*, *Rumex obtusifolius*, *Solanum nigrum* and *Urtica dioica* and in partial also *Mercurialis perennis*, *Adoxa moschatellina* and *Aconitum lycoctonum*.

Among the shrub species a good indicator of nitrogen is elder (*Sambucus nigra*). Its spreading in spruce monocultures was already studied by Rambousková (1984). Changes in its presence are hardly measurable in terms of its relationship to nitrogen, because in the course of improvement felling this species was man-made eliminated in many

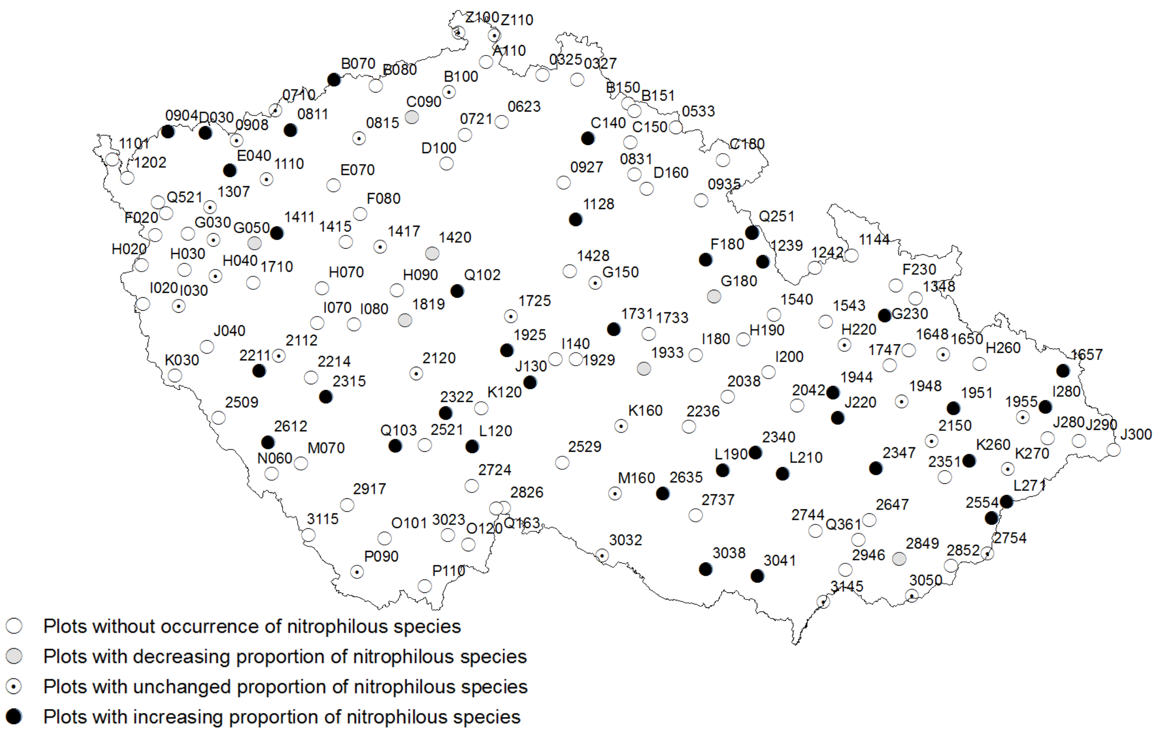

Fig. 5 Distribution of plots according to trends of the occurrence of nitrophilous species

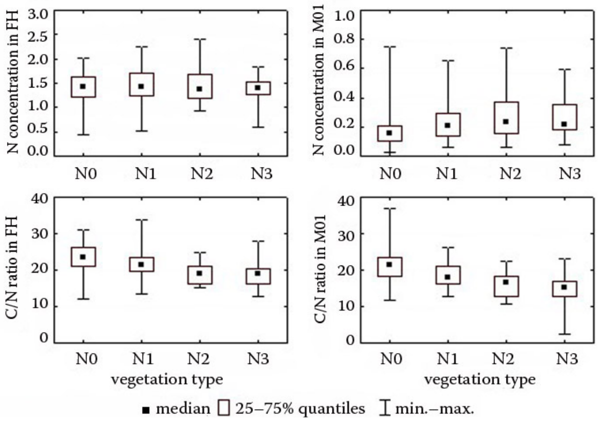

Fig. 6. Box plot – concentration of nitrogen and C/N ratio in FH and M01 (0–10 cm) layers according to the occurrence of nitrophilous species

N0 – plots without occurrence of nitrophilous species, N1 – plots with scarce occurrence of nitrophilous species, N2 – plots with significant occurrence of nitrophilous species with low coverage, N3 – plots with significant occurrence of nitrophilous species with high coverage ≥ 5%

plots. In the assessment of vegetation carried out usually in the summer aspect its occurrence is only sporadic and, in dry years, it may not be detected at all during this period.

Amongst these species, it was necessary to focus on those that are considerable indicators of increased nitrogen content in the soil and, at the same time, also occur at multiple sites, i.e. *Urtica dioica*, *Geranium robertianum*, *Impatiens parviflora* and *Alliaria petiolata*. A suitable and relatively frequently occurring species would also be cleavers (*Galium aparine*), which, however, is an annual plant. It occurs on a large scale, mainly in the spring aspect and, in the event of sufficient moisture, sometimes also in the autumn aspect and it is subject to seasonal fluctuation, depending on the weather. Out of 154 plots, the presence of these selected nitrophilous indicators was identified at a total of 74 plots. At 39 of these sites this represented a rather sporadic occurrence (1–2 species with coverage of up to 0.5% – N1). At 13 sites the occurrence of nitrophilous species was evaluated as significant (usually more species, though with relatively lower coverage of up to 5% – N2). At 22 sites there was a significant occurrence of nitrophilous species with high coverage (above 5% – N3). At other sites the occurrence of nitrophilous species was not recorded (N0) (Fig. 4). Based on the data from repeated investigations it was found that, at a total of 38 sites, the presence of nitrophilous species increased more or less during the last 15 years. A decline of the nitrophilous species occurrence was recorded at seven plots only (Fig. 5).

Based on the results it can be said that the higher occurrence of nitrophilous vegetation was found mostly at lower altitudes. The situation is similar to the spreading of invasive species, where the level of invasion decreases with altitude (Pyšek et al. 2012), which can be explained by the higher stability of mountainous habitats. Also according to Pyšek et al. (2012) the highest invasive species density as well as the highest level of invasion in forest communities are found in warm lowlands, especially in southern Moravia and central and eastern Bohemia.

The higher proportion of nitrophilous vegetation was recorded in southern Moravia and central Bohemia (Fig. 4). This can partly be explained by higher traffic volumes in the relatively densely populated areas with large urban conglomerations such as Prague and Brno, and also relatively lower altitude and absence of mountain areas.

When comparing groups of N0–N3 sites, i.e. in accordance with the occurrence of nitrophilous species, there were no statistically significant differences in nitrogen concentration (testing the humus and the mineral soil at a depth of 0–10 cm). When testing the C/N ratio, which provides a better evidence of the ecosystem nitrogen saturation, statistically significant differences in both horizons (FH – forest humus, 0–10 cm) at a significance level $\alpha = 0.05$ have already manifested (Fig. 6). As regards the species, in most cases there was an increase in the coverage of impatiens (*Impatiens parviflora*), while at some sites the presence of geranium (*Geranium robertianum*) and nettle (*Urtica dioica*) also increased.

When analysing the differences in the concentration of nitrogen in the soil and in the C/N ratio (for the FH and M01 horizons, i.e. 0–10 cm and for the M12 horizon, i.e. 10–20 cm) both at sites with and without the occurrence of selected nitrophilous species, significant statistical differences were found in the case of the C/N ratio across all the four nitrophilous species tested (*Geranium robertianum, Impatiens parviflora, Sambucus nigra, Urtica dioica*). In most cases significance was confirmed at the level $\alpha = 0.001$. The p values for both the non-parametric tests performed (Kruskal-Wallis ANOVA and median tests) and for all the three horizons and for the four selected nitrophilous species are listed in Table 1. As regards the concentration of nitrogen, statistically

Table 1. *P*-values of Kruskal-Wallis ANOVA and median tests for all the three horizons and for the four selected nitrophilous species

Species (number of plots with/without species)		FH		M01		M12	
		N_{tot}	C/N ratio	N_{tot}	C/N ratio	N_{tot}	C/N ratio
Geranium robertianum (24/121)	Kruskal-Wallis ANOVA	0.2050	0.0003	0.0739	0.0001	0.0066	0.0001
	median test	0.1923	0.0001	0.1683	0.0001	0.0231	0.0004
Impatiens parviflora (30/115)	Kruskal-Wallis ANOVA	0.6220	0.0058	0.5713	0.0012	0.2375	0.0006
	median test	0.9662	0.0447	0.3884	0.0447	0.2032	0.0003
Sambucus nigra (33/112)	Kruskal-Wallis ANOVA	0.3197	0.0445	0.2328	0.0005	0.2328	0.0002
	median test	0.3445	0.0329	0.1523	0.0009	0.5226	0.0002
Urtica dioica (50/95)	Kruskal-Wallis ANOVA	0.6927	0.0000	0.0110	0.0000	0.0006	0.0015
	median test	0.7724	0.0000	0.0122	0.0002	0.0004	0.0006

FH – humus layer, M01 – mineral soil layer 0–10 cm, M12 – mineral soil layer 10–20 cm, N_{tot} – total nitrogen content, C/N ratio – carbon to nitrogen ratio

significant differences were found at sites with and without nitrophilous species; in the case of *Geranium robertianum* at a depth of 10–20 cm and in the case of *Urtica dioica* in both the mineral layers tested – see the *P*-values in Table 1. Detected differences for elder (*Sambucus nigra*) are represented graphically in Fig. 7.

DISCUSSION

At most of the plots changes of two types have been observed. A number of changes in the occurrence and coverage of individual species can be evaluated as short-term seasonal fluctuations caused by different weather conditions from year to year. This statement is based on some additional assessments carried out in the approximately same period in the following year after the regular monitoring year. The most significant changes are reflected in the coverage of annual or biennial species, such as *Impatiens parviflora* and *Galium aparine*. These species require favourable moisture conditions at the time of their germination. During the dry season these species are virtually unable to germinate, and therefore, in some years, they may temporarily disappear from the phytosociological records. In the case of perennial plants, large seasonal fluctuations occur in regard to sorrel (*Oxalis acetosella*). There are also large differences in the coverage of the moss layer species, for which a high level of dependence on rainfall was confirmed.

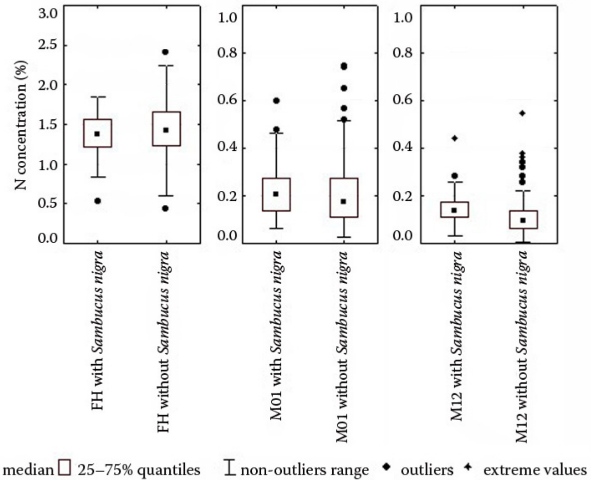

Fig. 7. Box plot – concentration of nitrogen in FH layer and in mineral layers (M01: 0–10 cm, M12: 10–20 cm) according to *Sambucus nigra* occurrence

In addition to these short-term temporary changes, longer-term changes and trends were also detected. Some of them are related to the development of vegetation and its successional changes. In stands of younger age classes, with a high level of tree canopy closure and poor light conditions, particularly in spruce monocultures, the herb layer is poorly developed. With increasing age, the thinning of stands usually is coming, in addition to the progressive development of the herb and moss layers. In mature stands with a low level of stocking after thinning and particularly in the case of the formation of clearings in the vicinity of the plots, there is usually a significant increase in the total coverage, the number of species and thereby also biodiversity of the ground vegetation.

Other changes are likely to be related to changes in the environment. The originally very high sulphur deposition has significantly decreased in central Europe during the 1990's. Nitrogen deposition, however, did not exhibit the same development and became the main source of acidification, replacing sulphur (Fottová 2003). Nitrogen still represents a considerable stress in many areas, the critical load for Central European coniferous forests is exceeded over a significant portion of the country (Zapletal 2006). Usual nitrogen bulk deposition varies from 9 to 15 kg·ha·yr^{-1}, the throughfall deposition in the most polluted area is still around 35 kg.ha.yr^{-1} (Boháčová et al. 2009). During the last 15 years, a higher proportion of nitrophilous species that prefer increased nitrogen content and of species that benefit from higher levels of other nutrients has been detected at some plots. Among the woody plants this is elder (*Sambucus nigra*), among herbs blackberry (*Rubus fruticosus* agg.), followed by *Urtica dioica, Geranium robertianum, Impatiens parviflora, Alliaria petiolata* and *Galium aparine*. At some plots the ecesis of clearcut species that frequently spread intensely in thinned forests and in younger age classes. The onset of a number of invasive alien species was also observed. These include an Asian *Impatiens glandulifera* and an American species that thrives in clearings, *Erechtites hieraciifolia*. The plots composed of non-native tree species and with different herb layer in comparison with the natural vegetation are especially prone to these changes. As regards the vegetation and the forest types, high-nutrient habitats (a high-nutrient or enriched series) are subject to the greatest change, while the acidophilous communities (an acidic series) appear to be more stable. Minimal changes were observed in the pine-oak forests with dominance of blueberry (*Vaccinium myrtillus*).

The effects of nitrogen on certain aspects of biodiversity have been monitored in Europe in recent years within the framework of the ICP Forests international programme and of other projects. Significant interaction was confirmed between the effects of nitrogen, other pollutants and climatic factors, especially in the case of oligotrophic epiphytic lichens (Giordani 2006). The tolerance level of lichens to nitrogen was utilised in recent years to assess the critical load of several forest ecosystems (e.g. Fenn et al. 2008). In accordance with the publication by Geiser et al. (2010), the critical load for lichens in coniferous forests of the northwest United States ranges between 0.7 and 4.4 kg·ha·yr^{-1}, depending on the rainfall. The conclusions of the European ForestBiota project, implemented at Level II sites, in which a value of 3.8 kg·ha·yr^{-1} is referred to, were similar (Giordani et al. 2011).

However, to demonstrate the direct effect of nitrogen deposition on the herbaceous ground vegetation is more complicated and the results are not so clear because the vegetation is more influenced by a number of additional factors, such as canopy closure, stand age, forest management, habitat conditions, geological substrate, climate, the life cycles of plants, the reserves of diasporas in the environment, etc. The effects of atmospheric deposition, particularly of sulphur and nitrogen, on vegetation and on its diversity were studied for a period of twelve growing seasons in the natural forests of Sweden (Qinghong, Bråkenhielm 1996), but the results were not statistically significant. The vegetation changes there are influenced by anthropogenic impacts to a far greater extent than they are by natural processes. It should be noted, however, that the overall level of sulphur and nitrogen deposition in Scandinavia is much lower than it is in Central and Western Europe. By contrast, numerous studies in Germany, in the Netherlands and in other countries in regard to the impact of eutrophication and acidification showed a significant effect of nitrogen deposition on species abundance and on the diversity of forest ecosystems (Tyler 1987; Thimonier et al. 1992; Schulze, Gerstberger 1993; de Vries 1995; van Dobben et al. 1999; Falkengren-Grerup, Schöttelndreier 2004). For example van Dobben and de Vries (2010), in one of their most recent studies, on the basis of analysis of Dutch and European ground vegetation monitoring data, came to the conclusion that atmospheric deposition has a relatively small, but statistically significant, effect on vegetation. High-level nitrogen deposition leads to a larger presence of nitrophilous species.

According to current knowledge concerning the Central European coniferous forests, the critical load is represented by the deposition of roughly 10 kg of nitrogen per hectare per year (Hofmeister et al. 2002). For deciduous forests, this value is somewhat higher (Bobbink et al. 2011). Exceeding this value significantly decreases the biodiversity of the forest because plant species that are sensitive to excess nitrogen are disappearing. This is just an indicative value; for some sensitive species the critical load is lower. For example, Swedish sources (Diekmann et al. 1999) indicate that the critical load for cranberry (*Vaccinium vitis-idaea*) is as low as 6 kg of nitrogen. If this limit is exceeded, cranberry will disappear for a long period. This empirical observation can also be confirmed in our forests. While in the 1950's cranberries were still relatively abundant in our forests, by the 1990's they had become almost a rarity and had been replaced by various species of nitrophilous grasses, for example (Hofmeister et al. 2002). The average value of the total nitrogen deposition in the Czech Republic is in the range of 10–20 kg of nitrogen per hectare per year and the critical load of nitrogen is exceeded in most of its area (Novotný et al. 2011).

The results of the assessment of the atmospheric deposition of nitrogen in the forest ecosystems in the Czech Republic show that while between the years 1994 and 2007 there was an overall decrease in the amount of NO_x deposition over a vast area, due to the reduction of NO_x pollution from large industrial sources, this effect was not applicable to the large cities nor to the vicinity of major roads where emissions from traffic are continuing to increase (Novotný et al. 2011). In these locations, we can expect a rather slight increase in the future, in relation to the increasing road traffic. Although an executive report of the ICP Forests programme from 2007 (Fischer et al. 2007) identifies a slight decrease in nitrogen deposition in Europe, the critical load was exceeded at two thirds of the monitoring sites, while in Central Europe, where there are still very high levels of depositions, exceeding the critical load occurring at almost all the sites where it was measured.

These findings are consistent with the conclusions reached in the framework of the ICP Forests international programme and with other studies. The processing of data from the assessment of ground vegetation at 477 European monitoring plots and data in regard to the level of nitrogen deposition confirmed that nitrogen deposition has an effect on changes in the species composition of the ground vegetation. It turns out that the number of plant species that indicate elevated nitrogen content has been increasing. Approximately 19% of the variability of vegetation can be explained in terms of deposition (Fischer et al. 2010).

CONCLUSIONS

The content of this paper is a basic evaluation of the data from the assessment at 154 monitoring plots pertaining to the ICP Forests programme of ground vegetation as a crucial factor of biodiversity. The goal was to record and document both the current typological and phytosociological characteristics of vegetation and its variations at the individual plots, and also its changes during the past 15 years in regard to the influence of nitrogen. At all the sites the typological and phytosociological characteristics of the vegetation were observed and their classification was defined as forest types or vegetation units, on the basis of the potential natural vegetation. Based on the data derived from the assessment of the ground vegetation and of the soil analyses, conducted at 154 plots, pertaining to the ICP Forests international programme, the dependence of the occurrence of and the coverage of the selected nitrophilous herbaceous indicators of nitrogen on the concentration of total nitrogen and on the C/N ratio in the humus layer and in the upper mineral soil horizon was analysed. At nearly a half of the plots the presence of the selected nitrophilous indicators was confirmed and at a number of plots their presence and coverage have increased in recent years. The differences between the sites in terms of the relationship between the occurrence and the presence of the selected nitrophilous species and the nitrogen content in the humus and the mineral layers were analysed. Statistically significant differences between the plots were detected in the humus and upper mineral horizons in regard to the C/N ratio. Significantly elevated concentrations of nitrogen were recorded in the 10–20 cm mineral horizon at sites where there was an occurrence of elder (*Sambucus nigra*). The results obtained were compared with results obtained abroad, in accordance with the same methodology at forest monitoring plots within the ICP Forests programme and with other studies with a similar focus.

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