

The use of Leaf Characteristics of Common Oak (*Quercus Robur* L.) to Monitor Ambient Ammonia Concentrations

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Abstract Biomonitoring of atmospheric ammonia (NH_3) concentrations is generally performed with epiphytic lichens, using species' abundances and/or nitrogen concentration as monitoring tools. However, the potential of leaf characteristics of trees to monitor the atmospheric NH_3 concentration has remained largely unexplored. Therefore, we performed a passive biomonitoring study with common oak (*Quercus robur* L.) at 34 sampling locations in the near vicinity of livestock farms, located in Flanders (northern

Belgium). We aimed at evaluating the potential of specific leaf area, leaf area fluctuating asymmetry, stomatal resistance, and chlorophyll content of common oak to monitor a broad range of NH_3 concentrations (four-monthly average of $1.9\text{--}29.9\text{ }\mu\text{g m}^{-3}$). No significant effects of ambient NH_3 concentration on the abovementioned leaf characteristics were revealed. Probably, differences in climate, soil characteristics, and concentrations of other air pollutants and/or genotypes confounded the influence of NH_3 . Consequently, this study demonstrates the inability of using these morphological, anatomical, and physiological common oak leaf characteristics to monitor ambient NH_3 concentration.

Keywords Passive biomonitoring · NH_3 concentration · Common oak · Specific leaf area · Fluctuating asymmetry · Relative chlorophyll content · Stomatal resistance

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1 Introduction

During the last decades, anthropogenic activities have led to an increased atmospheric concentration of reactive nitrogen (N) (Krupa 2003), which includes ammonia (NH_3), ammonium (NH_4^+), nitrogen oxide (NO_x), nitrous oxide (N_2O), nitrous acid (HNO_2), nitric acid (HNO_3), and organic N compounds. Reduced N (NH_x , i.e., NH_3 and NH_4^+) originating from intensive stockbreeding, is mainly responsible

for large-scale eutrophication and acidification (Krupa 2003; Pitcairn et al. 2003). Almost 30 % of the emitted NH_3 is converted to NH_4^+ , which is then either removed by wet or dry deposition (Krupa 2003; Paoli et al. 2010). Unaltered NH_3 is deposited in the vicinity of the source, leading to a trend of decreasing atmospheric NH_3 concentration away from the source (Frati et al. 2007; van Herk et al. 2003).

Atmospheric NH_3 is a major N source, increasing growth in N limited habitats (Krupa 2003). For example, high NH_3 concentrations acted as a nutrient for *Brassica oleracea* ($2.8 \text{ mg NH}_3\text{m}^{-3}$; Castro et al. 2008) and poplar (0.1 mgm^{-3} ; van Hove et al. 1989). However, NH_3 can also be phytotoxic when the plant's capacity of detoxification is exceeded, causing acute or chronic damage. Acute damage is reflected in bleached gray foliage, reduced growth, and even necrosis of leaf tissue (Sheppard et al. 2008; van der Eerden 1982). On a longer time scale, high NH_3 concentrations and consequently high NH_x deposition can cause chronic damage, such as ecosystem N saturation with enhanced N leaching to ground water (Gundersen et al. 2006), increased NO emission (Fenn et al. 1996), and a shift in species composition from N sensitive species (e.g., mosses) to nitrophilic species (e.g., some graminoids) (van der Eerden et al. 1998; Pitcairn et al. 2003). The degradation of freshwater, estuarine, and coastal marine ecosystems (Camargo and Alonso 2006) is also indicated as a consequence of increased NH_3 emission.

In order to minimize these negative effects of NH_x on ecosystems, NH_3 emission abatement policies were developed and pollution control techniques were applied. Dietary manipulation, storage, land application, and fertilizer substitution measures are considered as possible abatement measures (Cowell and Apsimon 1998; Olivier et al. 1998). To quantify the contribution of these control techniques in reducing the NH_3 emission, the critical level concept for environmental protection has been developed (Pitcairn et al. 2003). Generally, an annual average critical level of $8 \text{ } \mu\text{g NH}_3\text{m}^{-3}$ ($\text{CLE}_{\text{NH}_3,8}$) is used (Krupa 2003), which requires determination of atmospheric NH_3 concentration. Unfortunately, NH_3 is not routinely measured by air quality monitoring stations or passive samplers. The use of bioindicators and/or bioaccumulators provides a less costly alternative to monitor the atmospheric NH_3 concentrations and can also show whether critical levels are exceeded (Pitcairn et al.

2003). As a consequence, there has been growing interest in biomonitoring of atmospheric NH_3 concentrations, leading to the use of several organisms, and particularly lichens, in biomonitoring studies. The increase in abundance of (strictly) nitrophilic lichens due to a rise in bark pH (Frati et al. 2007), caused by an increased NH_3 concentration, makes them a suitable bioindicator. Moreover, the high potential of lichens for nutrient uptake due to the absence of roots and cuticle makes them a suitable bioaccumulator (Loppi and Nascimbene 2010).

Biomonitoring studies also frequently use tree leaf characteristics, such as chlorophyll fluorescence (Bortier et al. 2001), leaf hydrophobicity (Kardel et al. 2012), specific leaf area, and stomatal characteristics (Wuytack et al. 2010, 2011), to obtain information about air quality. However, these studies mainly deal with the biomonitoring of, e.g., ozone (O_3), NO_x , sulfur dioxide (SO_2), or heavy metals. Only a few studies have investigated the relationship between the NH_3 concentration and tree or leaf characteristics such as visible leaf injury (Van der Eerden et al. 1991), stomatal conductance (van Hove et al. 1989), erosion of the epicuticular wax layer, and growth of trees (Dueck et al. 1990). To our knowledge, an assessment of the potential of anatomical, morphological, and physiological tree leaf characteristics for biomonitoring of atmospheric NH_3 concentrations has not yet been reported. Therefore, the aim of this study was to assess the relationship between the four-monthly mean NH_3 concentration and leaf characteristics, i.e., specific leaf area (SLA), fluctuating asymmetry (FA), relative chlorophyll content (RCC), and stomatal density (SD) and pore surface (SPS) of common oak (*Quercus robur* L.). We hypothesized that SLA, FA, and SD would increase with increasing NH_3 concentration (Velickovic and Perisic 2006; Wuytack et al. 2010; 2011), while RCC and SPS would decrease (Joshi and Swami 2009).

2 Material and Methods

2.1 Study Area

The study was conducted in Flanders, northern Belgium (between 51° and 60° N, and 2.60° and 5.8° E; with a population density of $456 \text{ inhabitants/km}^2$), which is characterized by important industrial areas located at

the harbor and dockland of Antwerp (north of Brussels; mainly petrochemical industries) and Ghent (northwest of Brussels; steelworks and car assembly). Flanders is crisscrossed with international highways (E17, E19, E34, E313, E314, and E403) and contains two regions with intensive livestock breeding, emitting high amounts of NH_3 , i.e., pig farms in the western part and pig and poultry farms in the north-eastern part of Flanders. In 2008, the mean NH_x concentration amounted to $2.95 \mu\text{gm}^{-3}$ in Belgium, which exceeded the mean $\text{NH}_3\text{--NH}_4^+$ concentration of Europe (EU27) ($1.41 \mu\text{gm}^{-3}$) (www.emep.int). However, in livestock breeding regions, the mean NH_3 concentration in 2008 was up to $8.26 \mu\text{gm}^{-3}$ (VMM 2009).

Agriculture is estimated to be responsible for 93 % of the total NH_3 emission in Flanders, mainly through manure processing, grazing, artificial manure, manure store, and ride out livestock manure (MIRA-T 2008). Next to this, industry and traffic (catalytic converters) account for 5 and 2 % of the total NH_3 emission, respectively (www.vmm.be).

2.2 Experimental Design

In 2008, a biomonitoring network was developed in the vicinity of livestock farms in Flanders to analyze the effectiveness of epiphytic lichens as a bio-indicator for atmospheric NH_3 concentration. The abundance of lichens on common oak (*Quercus robur* L.) and hybrid poplar (*Populus × canadensis* Moench) was determined and related with the distance from the livestock farms, which was used as a proxy for the NH_3 concentration (Van den Broeck et al. 2009). The network covered 144 locations with 96 locations on sandy soils and 48 on loamy soils, characterized by a different NH_3 load. Locations were selected at more than 20 km of the North Sea coast, to avoid the influence of sea spray. At 100 locations, the monthly NH_3 concentration was measured from January 2008 till January 2009 with diffusive Radiello samplers (polyethylene cartridge impregnated with phosphoric acid). They were installed in partly open shelters at a height of 2.5–3 m on the north-eastern side of the trees to prevent contamination by wet deposition of NH_4^+ , since the main wind direction in the region is southwest (Van den Broeck et al. 2009). From this biomonitoring network, we selected 34 locations (Fig. 1) that were spread over Flanders and over the range of NH_3 concentrations, to perform a passive biomonitoring

study (using organisms present in the ecosystem) with common oak. Common oak is a soil vague species and is widely distributed in nature, which makes this species potentially interesting for biomonitoring. Only the sandy and sandy loam soil types, as determined from soil maps (geovlaanderen.be), were included in this selection to minimize the possible confounding effect of soil type. Soil was covered with grass vegetation at all sampling locations. The NH_3 concentration measured from April until July 2008 was considered as a representative measure of the NH_3 concentration during our fieldwork period (April until July 2009). At each location, fully expanded terminal leaves of six southerly orientated second-order branches of maximum three adjacent trees (<5 m distance) were sampled. We used the tree to which the Radiello sampler was attached, and, if possible, the trees left and/or right from the Radiello sampler tree were sampled. Subsequently, morphological (specific leaf area and fluctuating asymmetry), anatomical (stomatal density and dimensions) and physiological (relative chlorophyll content) leaf characteristics were determined on leaves of second order branches, at the end of July 2009.

2.3 Data Acquisition

2.3.1 Morphological Leaf Characteristics

Per branch we collected five fully developed and undamaged leaves to calculate specific SLA ($n=30$ per sampling location). From each leaf, two leaf discs (0.623 cm^2) were punched out at both sides of the midrib and in the middle of the leaf. The leaf discs were dried (48 h at 70°C) and weighed (B310S, Sartorius, Germany; $\pm 0.001 \text{ g}$), and SLA ($\text{cm}^2 \text{g}^{-1}$) was calculated per leaf as the ratio of area to dry weight of the discs.

The FA is defined as a slight, nondirectional, deviation from perfect symmetry of a bilateral character, due to genetic and/or environmental stress (Palmer and Strobeck 1986). To calculate leaf area FA, we randomly collected ten fully developed and undamaged leaves per branch ($n=60$ per sampling location). After harvest, each leaf was sliced along the middle of the mid vein and the surface area (cm^2) of both right (FA_{right}) and left (FA_{left}) lamina sides were measured using a leaf area meter (Li-3100, Li-COR, Nebraska, $\pm 0.01 \text{ cm}^2$). The FA_{right} and FA_{left} were used

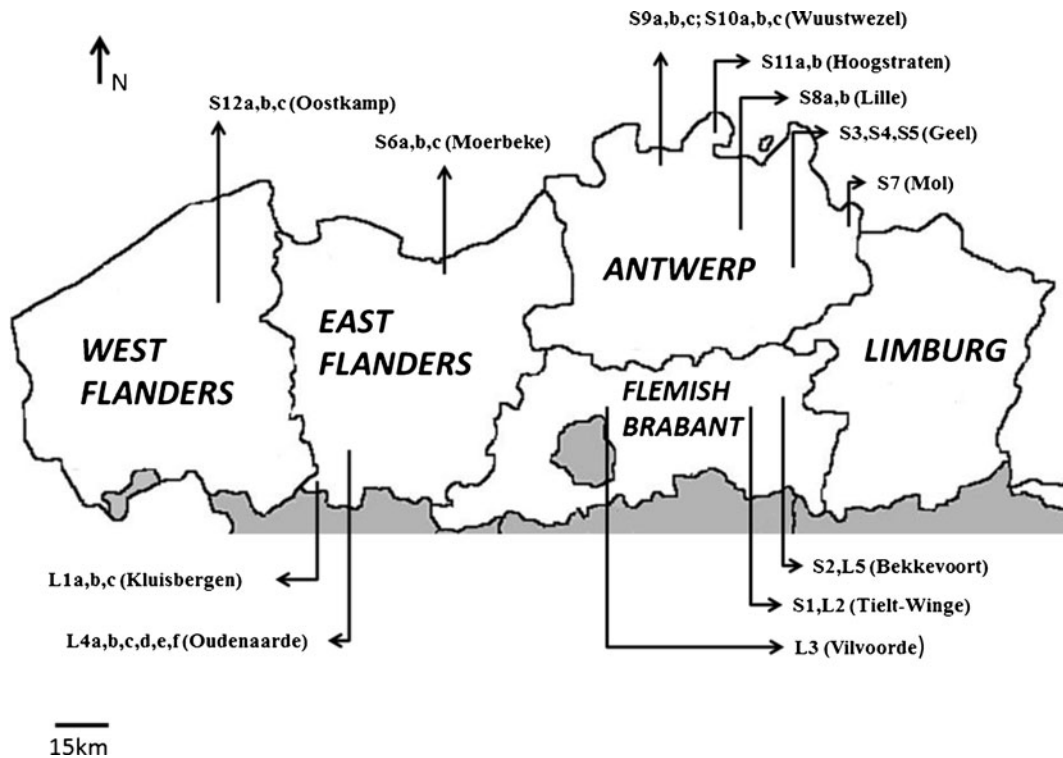


Fig. 1 Location of the sampling areas in Flanders, indicated by *S* (sandy soil) and *L* (sandy loam soil) and followed by a *number*, with one or more sampling locations in each area, indicated by a *letter*

to calculate FA (Wuytack et al. 2011). To test the precision of the measurements, FA_{right} and FA_{left} were each measured ten times for 25 randomly collected leaves, on different dates and in random order to reduce bias. Preliminary analyses were performed as described by Wuytack et al. (2011) to assess (1) the presence of directional asymmetry (DA) or antisymmetry (AS), (2) the presence of size dependency, and (3) the degree of measurement error.

2.3.2 Anatomical and Physiological Leaf Characteristics

Since common oak has hypostomatous leaves, stomatal characteristics were determined at the left and right abaxial leaf side of six fully developed and undamaged leaves at each location ($n=12$ per sampling location). Stomatal imprints, obtained as described by Wuytack et al. (2010), were analyzed with a light microscope (Wild Leitz GmbH 020-505.030 CX41RF, Olympus, Germany) connected with a camera at a magnification of 40×10 and imaging software (CellD, Imaging Software, Olympus, Germany). The number of stomata

was counted on a statistically determined number (N_{min} , see Wuytack et al. 2010) of microscopic fields to calculate the stomatal density (i.e., the number of stomata/ mm^2 leaf area; SD) on the left (SD_{left}) and right (SD_{right}) leaf side. Furthermore, stomatal length (SL; μm) and width (SW; μm) were measured to calculate the stomatal pore surface (i.e., the surface area of a widely opened stomatal pore, in μm^2 ; SPS) on the left (SPS_{left}) and right (SPS_{right}) leaf side. The values of SL, SW, and SD were used to calculate the theoretical stomatal resistance (R_s , in sm^{-1}) on the left ($R_{s,\text{left}}$) and right ($R_{s,\text{right}}$) leaf side (Olyslaegers et al. 2002).

Finally, on each branch, we collected four fully developed and undamaged leaves to measure RCC on both left and right leaf side ($n=48$ per sampling location). The leaves were washed with distilled water to remove small particles and air dried, after which RCC was immediately measured using a CCM-200 plus Chlorophyll Content Meter (Opti-Sciences, ADC Bioscientific). The CCM-200 has the advantage of being rapid, nondestructive, and pocket portable. Moreover, according to Cate and Perkins (2003), RCC values are strongly correlated with

chlorophyll concentrations determined by means of a spectrophotometer.

2.3.3 Growth Parameters

Circumference (m) at breast height (1.3 m) and tree age (year) were measured to take the possibly confounding effect of growth differences into account. To determine age, the trees were cored using a Pressler corer at the trunk base to obtain two perpendicular core samples per tree. Circumference ranged from 0.58 to 2.48 m and tree age ranged from 16 to 89 years. Since circumference and age were significantly correlated ($p < 0.001$, $R^2 = 0.53$), the number of variables was reduced using the ratio of circumference to age (growth rate, cm year^{-1}).

2.3.4 Air Quality Data

Within 1 week after exposure, NH_3 samplers were desorbed with ultrapure water that was analyzed using spectrophotometry. Air concentrations were calculated from the ion amounts in the desorption water using a temperature-dependent diffusivity based on a laboratory validation (Swaans et al. 2005). Regarding the precision of the samplers, the coefficient of variation between duplicate biweekly measurements at nine sites in 2005 was on average 3.3 % (Staelens et al. 2012).

In 2008, the monthly NH_3 concentration was highest during February and May and lowest during November and December (Fig. 2). This variation is caused by the fact that fertilization of agricultural fields is prohibited in the study region from 1 September to 15 February so that much fertilizer is applied at the end of February. In addition, during May, fields are fertilized before sowing of maize and after mowing of grass. The critical level for NH_3 of $8 \mu\text{g m}^{-3}$ was exceeded in the locations Wuustwezel and Hoogstraten (in the north, Fig. 1) and Oostkamp (in the west, Fig. 1), with a maximum exceedance of $16.53 \mu\text{g m}^{-3}$ in Wuustwezel (S10a; Fig. 1). To describe the NH_3 pollution at each location, the mean NH_3 concentration from April (start of growing season) till July 2009 (harvest) was calculated (Appendix).

2.4 Statistical Analysis

Because of the significant positive correlation ($p < 0.001$; $n = 204$) between SD_{left} and SD_{right} ($R^2 = 0.59$), SPS_{left} and $\text{SPS}_{\text{right}}$ ($R^2 = 0.54$) and $R_{\text{S left}}$ and $R_{\text{S right}}$ ($R^2 = 0.46$) and the significant negative correlation ($p < 0.001$; $n = 204$)

between the mean R_{S} and the mean SD ($R^2 = 0.56$) and SPS ($R^2 = 0.58$), only the mean R_{S} per leaf was used in the statistical analysis (Appendix). Mean SD ranged from 409 to 566 stomata/mm^2 , and mean SPS ranged from 50.3 to $109.4 \mu\text{m}^2$. Similarly, RCC_{left} and $\text{RCC}_{\text{right}}$ were positively correlated ($p < 0.001$; $n = 805$) so that the mean RCC per leaf was used in further analysis (Appendix).

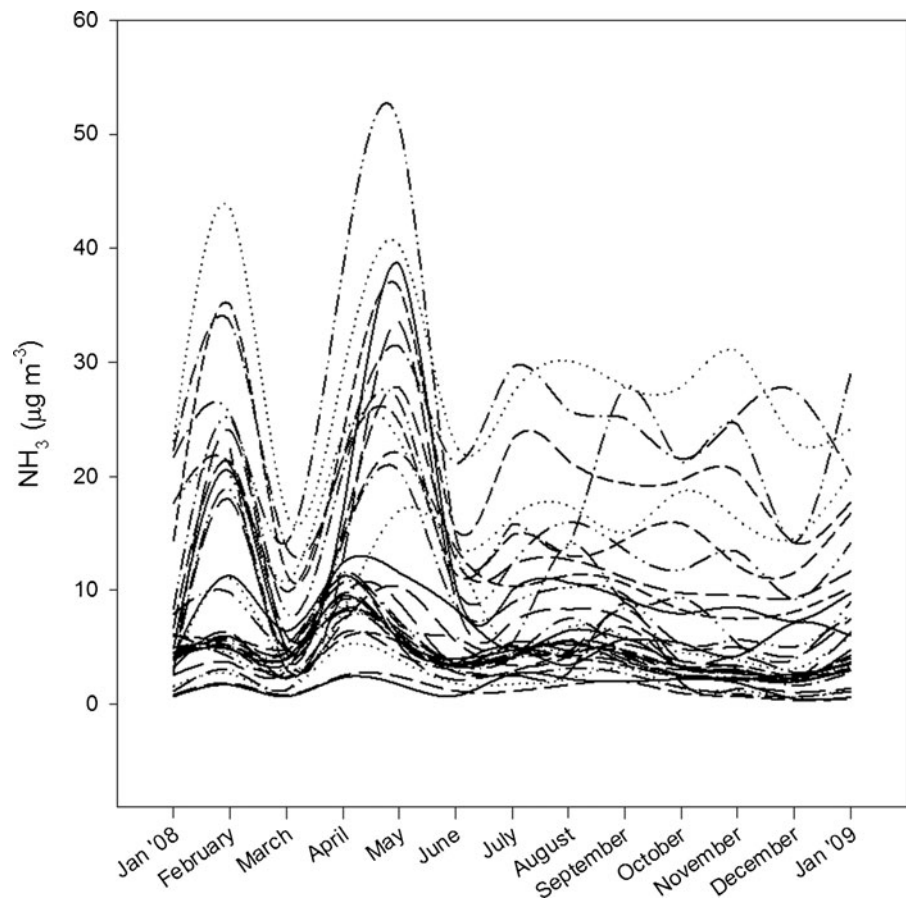
Several leaves were sampled on the same branch, multiple branches occurred on a tree, and multiple trees were analyzed per location. Because of this hierarchical nature of the data, we used linear mixed models to relate the leaf characteristics to the set of explanatory variables, i.e., NH_3 concentration, soil type, growth rate, NH_3 concentration \times soil type and NH_3 concentration \times growth rate. We first determined the optimal random model structure by stepwise deleting the lowest hierarchical level (starting with “branch”) and comparing the model with and without the deleted random effect using a likelihood ratio test. A linear model is preferred when only the level “location” remains and when the Akaike Information Criterion (AIC) value is lower for the linear model compared to the mixed effect model. Next, the fixed effects structure was optimized starting from a model that included all explanatory variables and the first order interactions with the NH_3 concentration. Model terms with non-significant ($p > 0.05$) parameter estimates and nonsignificant contributions to the overall model (likelihood ratio test) were successively removed, starting with the interaction terms. The null model is taken as the optimal fixed effects model when no explanatory variables contribute significantly to the overall model. All analyses were performed on the 5 % level of significance and run with R 2.10.1 (R Development Core Team, 2009) using the nlme package (Pinheiro et al. 2009) to fit the linear mixed models.

3 Results

3.1 Morphological Leaf Characteristics

For SLA, the optimal random model consisted of the level “location” and “tree” (Table 1). The SLA ranged from 80 to $188 \text{ cm}^2 \text{ g}^{-1}$ (Fig. 3a) and amounted to $154 \pm 20 \text{ cm}^2 \text{ g}^{-1}$ in the least NH_3 -polluted area (Flemish Brabant, L2, Fig. 1) and to $103 \pm 14 \text{ cm}^2 \text{ g}^{-1}$ in the most NH_3 -polluted area (Antwerp, S10a). The fixed effect model was optimized by first removing the interaction term “growth rate \times NH_3 concentration,” since it did not significantly

Fig. 2 Monthly NH_3 concentration ($\mu\text{g m}^{-3}$) from January 2008 to January 2009, for all the sampling locations



contribute to the model ($p=0.321$, $\chi^2=0.985$). On the contrary, the interaction term “soil type \times NH_3 concentration” improved the model ($p=0.018$), indicating a counteracting effect of “soil type.” However, the significant contribution of this interaction term in the fixed effect model was caused by a leverage effect, caused by two locations on loamy sand, L2 and L5 (Fig. 1). Therefore, these two locations were removed, and the statistical analysis was redone. The optimal random model still consisted of the level “location” and “tree” (Table 1). Variability between locations and trees explained 56 and 12 %, respectively, of the total variability of SLA. Variability within a tree (level “branch”), which can be seen as a large part of the residual variability (32 %), explained 24 % of the total variability. The optimal fixed effect model coincided with the null model (Table 1).

For FA, first of all, the presence of directional asymmetry, antisymmetry, and size dependency needed to be investigated. The one-sample t test revealed no significant difference ($p=0.336$) between left (LA) and right (RA) leaf area, indicating a lack of directional asymmetry. The

Shapiro–Wilk test revealed that the (R–L) distribution of leaf area significantly deviated from normality ($p<0.001$) and the positive kurtosis ($\gamma=3.437$) revealed a leptokurtic distribution, indicating a lack of antisymmetry. Unsigned (RA+LA)/2 values positively correlated with size trait (RA+LA)/2 ($r=0.500$, $p<0.001$, $n=1,928$). Therefore, we log-transformed the raw data of RA and LA and regressed $|\log \text{RA} - \log \text{LA}|$ on $(\log \text{RA} + \log \text{LA})/2$ to examine negative size dependency. Based on the slope of the regression (-0.0804) and the poor fit ($R^2=0.0005$), we concluded that the log transformation of the raw data caused no negative size dependency, and therefore, FA can be calculated by $|\log \text{RA} - \log \text{LA}|$. The precision of the measurements was tested by repeated measurements of leaf area of 25 leaves, resulting in a within-subject variability (σ^2) of 0.0059 for LA and 0.0037 for RA and a between-subject variability of 0.257. Moreover, significant relations were present between the measurement series ($R^2=0.814$ to 1.000 for LA and $R^2=0.984$ to 1.000 for RA; $p<0.001$), demonstrating high repeatability and reliability of the leaf area measurements.

Table 1 The contribution of each level (branch and tree) in the optimal random model and the contribution of the explanatory variables (single and interaction) in the optimal fixed effectmodel indicated by the p and χ^2 or F value for specific leaf area (SLA), fluctuating asymmetry (FA), relative chlorophyll content (RCC) and stomatal resistance (R_s)

	Explanatory variable	SLA ^a (cm ² g ⁻¹)	FA ^a (-)	RCC	R_s (sm ⁻¹)
Random model	Branch	$p=1.000$ $\chi^2=3.64 \times 10^{-9}$		$p<0.001$ $\chi^2=261.85$	$p=0.999$ $\chi^2=5.81 \times 10^{-8}$
	Tree	$p=0.0192$ $\chi^2=5.48$		$p<0.001$ $\chi^2=107.51$	$p<0.001$ $\chi^2=32.28$
Fixed effect model	Growth rate x NH ₃ concentration	$p=0.791$	$p<0.001$ $F=2.00$	$p=0.589$	$p=0.630$
	Soil type x NH ₃ concentration	$\chi^2=0.469$	$p=0.103$ $F=2.66$	$\chi^2=1.06$	$\chi^2=0.23$
	Soil type	$p=0.145$	$p=0.989$ $F=2 \times 10^{-4}$	$p=0.892$	$p=0.666$
	Growth rate	$\chi^2=5.399$	$p<0.001$ $F=11.82$	$\chi^2=0.618$	$\chi^2=1.57$
	NH ₃ concentration		$p<0.001$ $F=9.73$		
Null model		$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$

Values in italics indicate a significant ($p<0.05$) effect^a After removing the leverage effect caused by two locations on sandy loam, L2 and L5

We used a linear model to analyze FA instead of a mixed model, based on the difference of 40.7 in AIC value. The FA differed significantly between the sampling locations ($p<0.001$; $t=17.423$) and ranged from 0.00025 to 0.34979. This difference was significantly related to the interaction terms “growth rate×NH₃ concentration” ($p<0.001$; $t=4.710$) and “soil type×NH₃ concentration” ($p=0.004$; $t=2.894$). But, again, the significant contribution of “soil type×NH₃ concentration” was achieved by a leverage effect, caused by the same two locations L2 and L5 on sandy loam as for SLA. Therefore, the statistical analysis was repeated after omitting these two locations. The results of the linear model showed a significant effect of the NH₃ concentration, growth rate, and the interaction term “growth rate×NH₃ concentration” (Table 1, Fig. 3b).

3.2 Anatomical and Physiological Leaf Characteristics

For the stomatal resistance (R_s), the optimal random model consisted of the level “location” and “tree.” The variability between the sampling locations explained a small, although significant, part of the total variability (<0.1 %), while the variability between trees and the residual variability amounted to, respectively, 52 and 47 % of the total variability. The R_s ranged from 18 to 25 sm⁻¹ and amounted to 21 sm⁻¹ in the least NH₃-polluted area (L2)

and to 20 sm⁻¹ in the most NH₃-polluted area (S10a). The optimal fixed effect model coincided with the null model (Table 1). No relationship was found between the mean NH₃ concentration and R_s (Fig. 3D).

For the RCC, the full random model was used as optimal random model (Table 1). The variability in RCC was mainly related to the level “location” (37 %) and “tree” (35 %), only 10 % of the variation was related to the level “branch.” The RCC varied from 7.575 to 36.5. The optimal fixed effect model coincided with the null model (Table 1), and although RCC differed significantly between the sampling locations, no explanatory variable could explain the variability in RCC (e.g., NH₃; Fig. 3c).

4 Discussion

4.1 Within and Between-Plant Variability

The total observed variability of SLA, relative chlorophyll content (RCC), and stomatal resistance (R_s) of common oak was explained by a relative high within-plant (24 % for SLA, 10 % for RCC, and 44 % for R_s) and between-plant variability (12, 35, and 52 %, respectively). In contrast, for FA only a small part of the variation was explained by the within-plant (1.4 %) and

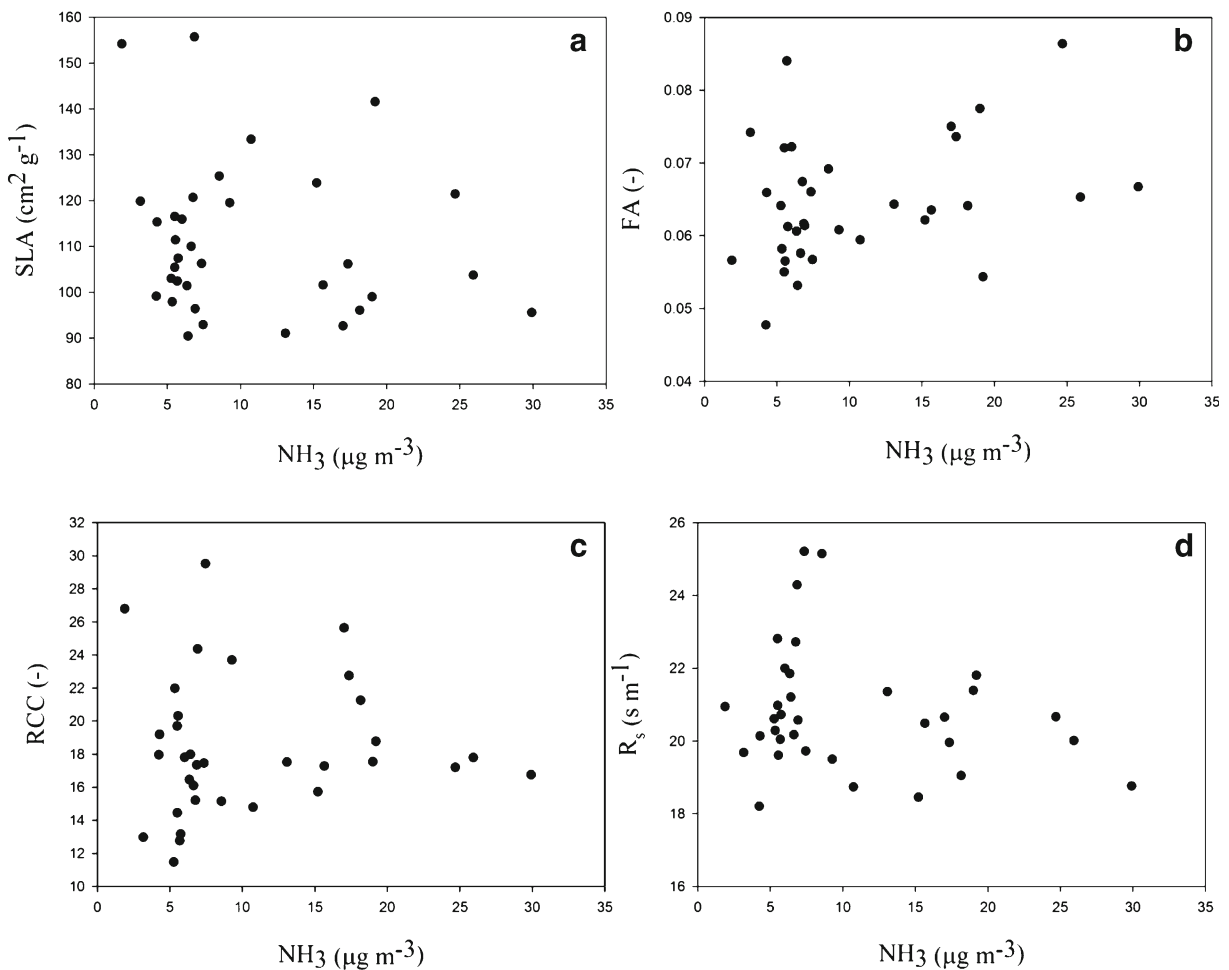


Fig. 3 Relationship between the mean NH_3 concentration and mean specific leaf area SLA (a), fluctuating asymmetry FA (b), relative chlorophyll content RCC (c), and stomatal resistance R_s (d)

between-plant (4.1 %) variability. The observed within-plant variability of SLA was low compared with the results of Poorter et al. (2009), where a twofold difference of SLA within a single plant was generally present. Within-plant variability of leaf characteristics can occur due to a gradient in light, air temperature, air humidity, and wind velocity, present in larger trees (Poorter et al. 2009 and references herein) and plant-specific factors, such as age, development stage, and the position of the leaf on the plant (Gunn et al. 1999). Leaves adapt to lower irradiance by producing thinner and larger leaves, resulting in a higher SLA (Wuytack et al. 2011), and by an increased R_s as a consequence of optimizing CO_2 uptake and reducing water loss by transpiration (Barber et al. 2004; Gratani et al. 2006). Moreover, sun leaves possess a higher chlorophyll content compared to shade leaves due to the high irradiance adaptation response of the

photosynthetic response apparatus (Sarijeva et al. 2007), while under too much light, leaves become chlorotic (Larcher 2003). High air temperature leads to a decrease in chlorophyll content not only in order to increase reflectance and decrease the intercepted light but also as a protection mechanism against photodestruction (Gratani et al. 2011). An increase in air temperature also causes a decrease in R_s up to the optimal air temperature, which can be seen as an adaptation to reduce evapotranspirational water loss (Beerling and Chaloner 1993; Barber et al. 2004). Furthermore, genetic differences between the sampled trees can lead to the observed between-plant variability as well. This genetic variability not only may underlie small differences in SLA (Bonser et al. 2010) and stomatal responses (Pääkkönen et al. 1993) but can also lead to larger differences of FA between clones than between treatments (Dimitriou et al. 2006).

4.2 Between-Site Variability

In case of FA, RCC, and R_S , the between-site variability explained a small part of the total variability (<0.1 %), while the between-site variability of SLA explained 56 % of the total variability. The between-site variability of FA was related to the interaction between growth rate and mean NH_3 concentration. However, this interaction is difficult to interpret, due to the continuous character of the two explanatory variables. Probably, as stated by Martel et al. (1999), the faster growing oaks developed a higher developmental instability due to an increased energy demand to produce larger leaves, which can give rise to an increased FA. However, regardless of the significant between-site variability, the low measurement error, and the high statistical power (91 %), the very high residual variability (95 %) raises the question whether FA needs to be interpreted as noise rather than as a true signal.

For all other leaf characteristics, the between-site variability was not related to the mean NH_3 concentration (Fig. 3a–d), even though the critical level of $8 \mu\text{g NH}_3\text{m}^{-3}$ was exceeded at several sampling locations. The incidence of such an exceedance may indicate that direct adverse effects on plants may occur, as stated by Posthumus (1988). However, no adverse effect of NH_3 on the measured leaf characteristics of mature common oak could be detected in this study. The lack of an adverse effect confirms the statement of Cape et al. (2009) that “exceedances of the critical level do not guarantee that an (adverse) effect will be observed, due to the presence of other environmental stressors and their interaction with NH_3 concentration.” Indeed, plants are exposed to a broad range of uncontrolled and/or unmeasured variables, which interact in an unknown synergistic or antagonistic way, making it difficult to separate the effects of intercorrelated variables (Wuytack et al. 2011). Therefore, biomonitoring studies need to take into account the possible single and interacting effects of other atmospheric pollutants, such as SO_2 , nitrogen dioxide (NO_2), and O_3 on SLA (e.g., Bassin et al. 2009), chlorophyll content (e.g., van Hove et al. 1992), FA (e.g., Wuytack et al. 2011), and stomatal characteristics (e.g., Elagoz et al. 2006). Atmospheric pollutants can disturb stomatal control mechanisms (Robinson et al. 1998), since plants optimize their stomatal closure efficiency by increasing stomatal density and decreasing stomatal pore surface as a response to air pollution (Elagoz et al. 2006; Kardel et al. 2010; Wuytack et al. 2010). Chlorophyll degradation can occur as a response to O_3 (Calatayud et al. 2011), power plant pollution (Sharma

and Tripathi 2009), and particulate matter (Kuki et al. 2008). Van Hove et al. (1991) also demonstrated that moderate NH_3 concentrations can alleviate the inhibitory effect of SO_2 on photosynthesis, indicating a synergistic interaction between NH_3 and SO_2 . In contrast, O_3 can increase the plant's sensitivity to NH_3 by decreasing the amount of energy available for NH_3 assimilation, indicating an antagonistic interaction between NH_3 and O_3 (Krupa 2003). However, the concentration of other air pollutants was not measured in our study at each sampling location, making it impossible to evaluate the share of these pollutants and their interactions in the significant between-site variability of the leaf characteristics of common oak. Additionally, passive biomonitoring with trees has the disadvantage that effects of soil characteristics (e.g., nutrient availability) cannot be accounted for. Nutrient availability can influence SLA by changing lamina and mesophyll thickness (Meziane and Shipley 1999). No information was available on other (a)biotic stressors that may have occurred in the past, such as historic management (e.g., pruning intensity), (mechanical) soil disturbances, and herbivore attacks and diseases. Mechanical soil disturbances can, for example, increase plant FA (Freeman et al. 2005). Herbivory can cause changes in the microclimate of the remaining foliage and increase the specific hydraulic conductance of the damaged leaves, leading to an increased stomatal conductance (Pataki et al. 1998). In addition, the metabolism of old trees is relatively buffered and extra ammonia absorbed can be only a small part of the whole N used by the trees. To avoid these confounding effects of passive biomonitoring, active biomonitoring (Wuytack et al. 2011), i.e., with organisms that are introduced in the ecosystem, can be performed instead.

Not only the presence of other environmental stressors but also the tolerance of common oak for NH_3 can help to explain the absence of adverse effects on the considered leaf characteristics. The sensitivity of different plant species to NH_3 exposure is listed by Krupa (2003), with common oak as intermediate susceptible for short-term exposures to high NH_3 concentrations. The sensitivity of oak to lower NH_3 concentrations over longer periods is not known. The high N availability in the soil due to high NH_x deposition near intensive livestock farms might increase the NH_4^+ pools and apoplastic pH in leaf tissue, causing an increased stomatal compensation point (Mattson and Schjoerring 2002) and, therefore, a lower direct absorption of potentially harmful NH_3 . It is also possible that the measured leaf characteristics are not

sensitive to the ambient NH_3 concentration, since leaf characteristics of a same tree can respond differently to ambient air pollution (Wuytack et al. 2011). Therefore, more biochemical and/or physiological leaf characteristics should be measured, such as ascorbate, glutathione, superoxide dismutase, and chlorophyll fluorescence—as they might reflect changes that cannot be detected at the anatomical or morphological level—before the suitability of a species as bioindicator can be correctly assessed. Tree ring analysis can also be considered to be used in bio-monitoring studies, as this might offer insight into (annual) pollution dynamics in the past.

5 Conclusions

Our results indicated that specific leaf area, fluctuating asymmetry, relative chlorophyll content, and stomatal resistance of common oak are not good bioindicators for monitoring four-monthly mean atmospheric NH_3 concentrations in the vicinity of livestock farms. Moreover, these leaf characteristics demonstrate a high within- and between-plant variability, which reflects a high leaf sensitivity and questions the effectiveness of common oak as a passive bioindicator. The lack of relationships between the studied leaf characteristics and the mean four-monthly NH_3 concentration can be caused by confounding effects of (1) other environmental factors, (2) genetic differences, (3) tree history in relation to human and natural disturbances, and (4) intermediate susceptibility of common oak, due to a possibly high stomatal compensation point for NH_3 . Therefore, we conclude that the use of an active bioindicator is more appropriate than the use of a passive bioindicator and that the measurement of other environmental factors, such as O_3 , SO_2 , and NO_x are necessary when performing a biomonitoring study. The use of an active bioindicator reduces the variability caused by genotypes and soil characteristics, and therefore, the effectiveness of several species as active bioindicator needs to be tested. In general, a lot of research is still necessary to evaluate the potential of trees as active or passive bioindicators.

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Appendix

Table 2 Mean NH_3 concentration (April until July 2008), specific leaf area (SLA), fluctuating asymmetry (FA), relative chlorophyll content (RCC) and stomatal resistance (R_s) for each sampling area, with one or more sampling locations in each area

Soil type	Sampling location	NH_3 ($\mu\text{g m}^{-3}$)	SLA ($\text{cm}^2 \text{g}^{-1}$)	FA	RCC	R_s (sm^{-1})
Sandy	S1	3.17	119.8	0.074	12.98	19.68
	S2	4.24	99.1	0.048	17.96	18.20
	S3	4.30	115.3	0.066	19.19	20.14
	S4	6.63	110.0	0.058	16.10	20.17
	S5	6.76	120.7	0.067	15.21	22.72
	S6a	6.85	155.7	0.062	17.35	24.29
	S6b	7.35	106.3	0.066	17.45	25.21
	S6c	8.56	125.4	0.069	15.14	25.15
	S7	6.91	96.4	0.061	24.36	20.57
	S8a	9.28	119.5	0.061	23.69	19.50
	S8b	10.74	133.4	0.059	14.79	18.73
	S9a	15.22	123.9	0.062	15.72	18.45
	S9b	13.08	91.0	0.064	17.52	21.35
	S9c	15.66	101.6	0.064	17.28	20.48
	S10a	25.93	103.8	0.065	17.79	20.01
	S10b	17.02	92.6	0.075	25.63	20.65
	S10c	19.00	99.0	0.077	17.53	21.39
	S11a	24.69	121.5	0.086	17.19	20.66
	S11b	17.36	106.2	0.074	22.75	19.96
	S12a	19.21	141.6	0.054	18.78	21.81
	S12b	18.16	96.1	0.064	21.25	19.05
	S12c	29.92	95.6	0.067	16.75	18.76
Sandy loam	L1a	6.34	101.4	0.061	16.46	21.85
	L1b	6.42	90.4	0.053	17.98	21.20
	L1c	5.69	102.5	0.084	12.77	20.04
	L2	1.89	154.2	0.057	26.78	20.94
	L3	5.35	97.9	0.058	21.98	20.29
	L4a	6.01	115.9	0.072	17.80	22.00
	L4b	5.51	116.5	0.072	14.45	20.98
	L4c	5.51	105.4	0.055	19.69	22.81
	L4d	5.27	103.0	0.064	11.47	20.61
	L4e	5.75	107.4	0.061	13.17	20.72
	L4f	5.57	111.4	0.057	20.31	19.61
	L5	7.45	92.9	0.057	29.52	19.72

References

- Barber, J. L., Thomas, G. O., Kerstiens, G., & Jones, K. C. (2004). Current issues and uncertainties in the measurement and modeling of air-vegetation exchange and within-plant processing of POPs. *Environmental Pollution*, 128, 99–138.
- Bassin, S., Werner, R. A., Sorgel, K., Volk, M., Buchmann, N., & Fuhrer, J. (2009). Effects of combined ozone and nitrogen deposition on the in situ properties of eleven key plant species of a subalpine pasture. *Oecologia*, 158, 747–756.
- Beerling, D. J., & Chaloner, W. G. (1993). The impact of atmospheric CO₂ and temperature-change on stomatal density-observations from *Quercus robur* Lammas leaves. *Annals of Botany*, 71, 231–235.
- Bonsler, S. P., Ladd, B., Keyne, M., Hall, M. D., & Forster, M. A. (2010). The adaptive value of functional and life-history traits across fertility treatments in an annual plant. *Annals of Botany*, 106, 979–988.
- Bortier, K., Vandermeiren, K., De Temmerman, L., & Ceulemans, R. (2001). Growth, photosynthesis and ozone uptake of young beech (*Fagus sylvatica* L.) in response to different ozone exposures. *Trees*, 15, 75–82.
- Calatayud, V., Cervero, J., Calvo, E., Garcia-Breijo, F. J., Reig-Arminana, J., & Sanz, M. J. (2011). Responses of evergreen and deciduous *Quercus* species to enhanced ozone levels. *Environmental Pollution*, 159, 55–63.
- Camargo, J. A., & Alonso, A. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environment International*, 32, 831–849.
- Cape, J. N., van der Eerden, L. J., Sheppard, L. J., Leith, I. D., & Sutton, M. A. (2009). Evidence for changing the critical level for ammonia. *Environmental Pollution*, 157, 1033–1037.
- Castro, A., Stulen, I., & De Kok, L. J. (2008). Atmospheric NH₃ as plant nutrient: a case study with *Brassica oleracea*. *Environmental Pollution*, 154, 467–472.
- Cate, T. M., & Perkins, T. D. (2003). Chlorophyll content monitoring in sugar maple (*Acer saccharum*). *Tree Physiology*, 23, 1077–1079.
- Cowell, D. A., & Apsimon, H. M. (1998). Cost-effective strategies for the abatement of ammonia emissions from European agriculture. *Atmospheric Environment*, 32, 573–580.
- Dimitriou, I., Aronsson, P., & Weih, M. (2006). Stress tolerance of five willow clones after irrigation with different amounts of landfill leachate. *Bioresource Technology*, 97, 150–157.
- Dueck, T. A., Dorel, F. G., Ter Horst, R., & Van der Eerden, L. J. M. (1990). Effects of ammonia, ammonium sulphate and sulphur dioxide on the frost sensitivity of Scots pine (*Pinus sylvestris* L.). *Water, Air, and Soil Pollution*, 54, 35–49.
- Elagoz, V., Han, S. S., & Manning, W. J. (2006). Acquired changes in stomatal characteristics in response to ozone during plant growth and leaf development of bush beans (*Phaseolus vulgaris* L.) indicate phenotypic plasticity. *Environmental Pollution*, 140, 395–405.
- Fenn, M. E., Poth, M. A., & Johnson, D. W. (1996). Evidence for nitrogen saturation in the San Bernadino Mountains in southern California. *Forest Ecology and Management*, 82, 211–230.
- Frati, L., Santoni, S., Nicolardi, V., Gaggi, C., Brunialti, G., Guttova, A., et al. (2007). Lichen biomonitoring of ammonia emission and nitrogen deposition around a pig stock-farm. *Environmental Pollution*, 146, 311–316.
- Freeman, D. C., Brown, M. L., Duda, J. J., Grarham, J. H., Emlen, J. M., Krzysik, A. J., et al. (2005). Leaf fluctuating asymmetry, soil disturbances and plant stress: a multiple year comparison using two herbs, *Ipomoea pandurata* and *Cnidioscolus stimulosus*. *Ecological Indicators*, 5, 85–95.
- Gratani, L., Covone, F., & Larcher, W. (2006). Leaf plasticity in response to light of three evergreen species of the Mediterranean maquis. *Trees-Structure and Function*, 20, 549–558.
- Gratani, L., Catoni, R., & Varone, L. (2011). Photosynthetic and leaf respiration activity of *Malcolmia littorea* (L.) R. Br. in response to air temperature. *Photosynthetica*, 49, 65–74.
- Gundersen, P., Schmidt, I. K., & Rauland-Rasmussen, K. (2006). Leaching of nitrate from temperate forests—effects of air pollution and forest management. *Environmental Reviews*, 14, 1–57.
- Gunn, S., Farrar, J. F., Collis, B. E., & Nason, M. (1999). Specific leaf area in barley: individual leaves versus whole plants. *New Phytologist*, 143, 45–51.
- Joshi, P. C., & Swami, A. (2009). Air pollution induced changes in the photosynthetic pigments of selected plant species. *Journal of Environmental Biology*, 30, 295–298.
- Kardel, F., Wuyts, K., Babanezhad, M., Vitharana, U. W. A., Wuytack, T., Potters, G., et al. (2010). Assessing urban habitat quality based on specific leaf area and stomatal characteristics of *Plantago lanceolata* L. *Environmental Pollution*, 158, 788–794.
- Kardel, F., Wuyts, K., Babanezhad, M., Wuytack, T., Adriaenssens, S., & Samson, R. (2012). Tree leaf wettability as passive bio-indicator of urban habitat quality. *Environmental and Experimental Botany*, 75, 277–285. doi:10.1016/j.envexpbot.2011.07.011.
- Krupa, S. V. (2003). Effects of atmospheric ammonia (NH₃) on terrestrial vegetation: a review. *Environmental Pollution*, 124, 179–221.
- Kuki, K. N., Oliva, M. A., Pereira, E. G., Costa, A. C., & Canbraia, J. (2008). Effects of simulated deposition of acid mist and iron ore particulate matter on photosynthesis and the generation of oxidative stress in *Schinus terebinthifolius* Radii and *Sophora tomentosa* L. *Science of the Total Environment*, 403, 207–214.
- Larcher, W. (2003). *Physiological plant ecology: ecophysiology and stress physiology of functional groups*. Germany: Springer. 513p.
- Loppi, S., & Nascimbene, J. (2010). Monitoring H₂S air pollution caused by the industrial exploitation of geothermal energy: the pitfall of using lichens as bioindicators. *Environmental Pollution*, 158, 2635–2639.
- Martel, J., Lempa, K., & Haukioja, E. (1999). Effects of stress and rapid growth on fluctuating asymmetry and insect damage in birch leaves. *Oikos*, 86, 208–216.
- Mattson, M., & Schjoerring, J. K. (2002). Dynamic and steady-state responses of inorganic nitrogen pools and NH₃ exchange in leaves of *Lolium perenne* and *Bromus erectus* to

- changes in root nitrogen supply. *Plant Physiology*, 128, 742–750.
- Meziane, D., & Shipley, B. (1999). Interacting determinants of specific leaf area in 22 herbaceous species: effects of irradiance and nutrient availability. *Plant, Cell & Environment*, 22, 447–459.
- Olivier, J. G. J., Bouwman, A. F., Van der Hoek, K. W., & Berdowski, J. J. M. (1998). Global air emission inventories for anthropogenic sources of NO_x, NH₃ and N₂O in 1990. *Environmental Pollution*, 102, 135–148.
- Olyslaegers, G., Nijs, I., Roebben, J., Kockelbergh, F., Vanassche, F., Laker, M., et al. (2002). Morphological and physiological indicators of tolerance to atmospheric stress in two sensitive and two tolerant tea clones in South Africa. *Experimental Agriculture*, 38, 397–410.
- Pääkkönen, E., Paasisalo, S., Holopainen, T., & Karenlampi, L. (1993). Growth and stomatal responses of birch (*Betula pendula* Roth) clones to ozone in open-air and chamber fumigations. *New Phytologist*, 125, 615–623.
- Palmer, A. R., & Strobeck, C. (1986). Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecological Systems*, 17, 391–421.
- Paoli, L., Pirintsos, S. A., Kotzabasis, K., Pisani, T., Navakoudis, E., & Loppi, S. (2010). Effects of ammonia from livestock farming on lichen photosynthesis. *Environmental Pollution*, 158, 2258–2265.
- Pataki, D. E., Oren, R., & Philips, N. (1998). Responses of sap flux and stomatal conductance of *Pinus taeda* L. trees to stepwise reductions in leaf area. *Journal of Experimental Botany*, 49, 871–878.
- Pinheiro, K., Bates, D., DebRoy, S., Sarkar, D., the R Core team (2009). nlme: linear and nonlinear mixed effects models. R package version 3.1-96.
- Pitcairn, C. E. R., Fowler, D., Leith, I. D., Sheppard, L. J., Sutton, M. A., Kennedy, V., et al. (2003). Bioindicators of enhanced nitrogen deposition. *Environmental Pollution*, 126, 353–361.
- Poorter, H., Niinemets, U., Poorter, L., Wright, I. J., & Villar, R. (2009). Tansley review: causes and consequences of variation in leaf mass area (LMA): a meta-analysis. *New Phytologist*, 182, 565–588.
- Posthumus, A. C., 1988. Critical levels for effects of ammonia and ammonium. Proceedings of the Bad Harzburg Workshop. UBA, Berlin, pp 117–127.
- Robinson, M. F., Heath, J., & Mansfield, T. A. (1998). Disturbances in stomatal behavior caused by air pollutants. *Journal of Experimental Botany*, 49, 461–469.
- Sarijeva, G., Knapp, M., & Lichtenthater, H. K. (2007). Differences in photosynthetic activity, chlorophyll and carotenoid levels, and in chlorophyll fluorescence parameters in green sun and shade leaves of *Ginkgo* and *Fagus*. *Journal of Plant Physiology*, 164, 950–955.
- Sharma, A. P., & Tripathi, B. D. (2009). Biochemical responses in tree foliage exposed to coal-fires power plant emission is seasonally dry tropical environment. *Environmental Monitoring and Assessment*, 158, 197–212.
- Sheppard, L. J., Leith, I. D., Crossley, A., van Dijk, N., Fowler, D., Sutton, M. A., et al. (2008). Stress responses of *Calluna vulgaris* to reduced and oxidized N applied under ‘real world conditions’. *Environmental Pollution*, 154, 404–413.
- Staelens, J., Wuyts, K., Adriaenssens, S., Van Avermaet, P., Buysse, H., Van den Bril, B., et al. (2012). Trends in atmospheric nitrogen and sulphur deposition in northern Belgium. *Atmospheric Environment*, 49, 186–196.
- Swaans, W., Damen E., Goelen E., De Fré R. (2005). Validation of Radiello NH₃ passive sampler (in Dutch). Study on the authority of the Flemish Environment Agency. Final report 2005/MIM/R/045, VITO.
- Van den Broeck, D., Herremans, M., Verbeylen, G., Jacobs, I., Dorsselaer, P. (2009). Korstmossen als bio-indicator voor ammoniakconcentraties (Eindrapport). Rapport 2009/5 Natuurpunt Studie, Mechelen, België (in Dutch).
- van der Eerden, L. J. M. (1982). Toxicity of ammonia to plants. *Agriculture and Environment*, 7, 223–235.
- van der Eerden, L. J. M., Dueck, T. A., Berdowski, J. J. M., Grevén, H., & Van Dobben, H. F. (1991). Influence of NH₃ and (NH₄)₂SO₄ on heathland vegetation. *Acta Botanica Neerlandica*, 40, 281–297.
- van der Eerden, L., De Vries, W., & Van Dobben, H. (1998). Effects of ammonia deposition on forests in the Netherlands. *Atmospheric Environment*, 32, 525–532.
- van Herk, C. M., Mathijssen-Spiekman, E. A. M., & de Zwart, D. (2003). Long distance nitrogen air pollution effects on lichens in Europe. *The Lichenologist*, 35, 347–359.
- van Hove, L. W. A., van Kooten, O., Adema, E. H., Vredenburg, W. J., & Pieters, G. A. (1989). Physiological effects of long-term exposure to low and moderate concentrations of atmospheric NH₃ on poplar leaves. *Plant, Cell & Environment*, 12, 899–908.
- van Hove, L. W. A., van Kooten, O., van Wijk, K. J., Vredenburg, W. J., Adema, E. H., & Pieters, G. A. (1991). Physiological effects of long term exposure to low concentrations of SO₂ and NH₃ on poplar leaves. *Physiologia Plantarum*, 82, 32–40.
- van Hove, L. W. A., Bossen, M. E., Mensink, M. G. J., & van Kooten, O. (1992). Physiological effects of a long term exposure to low concentrations of NH₃, NO₂ and SO₂ on Douglas fir (*Pseudotsuga menziesii*). *Physiologia Plantarum*, 86, 559–567.
- Velickovic, M., & Perisic, S. (2006). Leaf fluctuating asymmetry of common plantain as an indicator of habitat quality. *Plant Biosystems*, 140, 138–145.
- VMM. (2009). ‘Acid rain’ in Flanders, deposition network acidification 2008. Erembodegem: Flemish Environmental Agency (in Dutch).
- Wuytack, T., Verheyen, K., Wuyts, K., Kardel, F., Adriaenssens, S., & Samson, R. (2010). The potential of biomonitoring of air quality using leaf characteristics of white willow (*Salix alba* L.). *Environmental Monitoring and Assessment*, 171, 197–204.
- Wuytack, T., Wuyts, K., Van Dongen, S., Baeten, L., Kardel, F., Verheyen, K., et al. (2011). The effect of air pollution and other environmental stressors on leaf fluctuating asymmetry and specific leaf area of *Salix alba* L. *Environmental Pollution*, 159, 2405–2411.