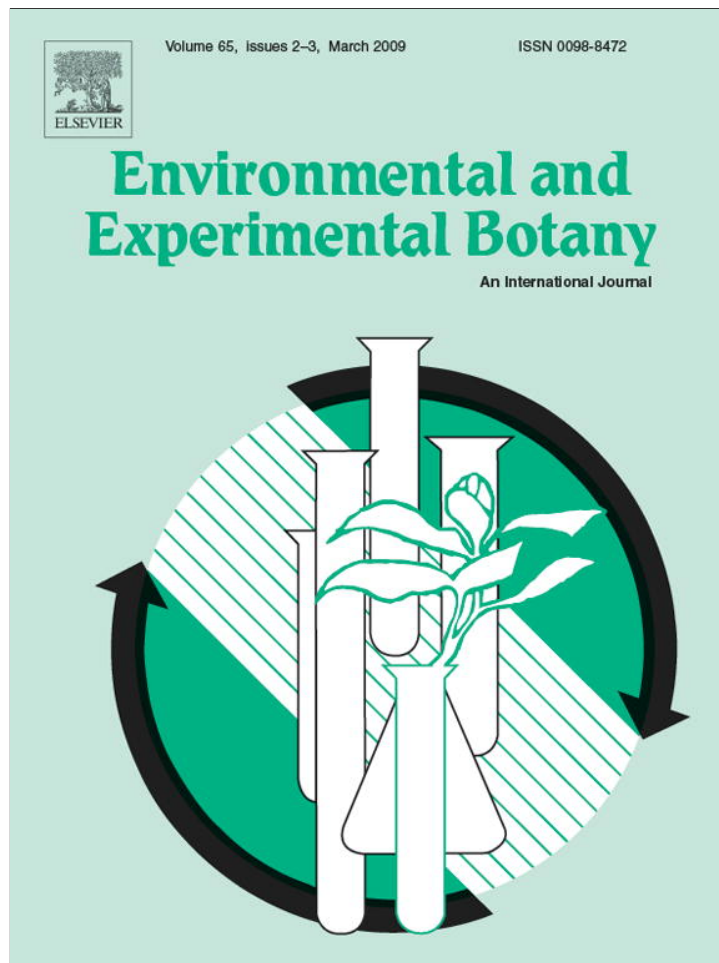


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## Biomonitoring of urban habitat quality by anatomical and chemical leaf characteristics

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### ABSTRACT

This study focused on the potential of specific leaf area, stomatal density and stomatal pore surface as easy-to-measure plant parameters in low cost biomonitoring of urban habitat quality with a high spatial resolution. The study area (81.5 km<sup>2</sup>) was the city of Gent, Belgium. In the study area 148 sampling locations were identified within 4 land use classes. Specific leaf area, stomatal density, stomatal pore surface, minimal stomatal resistance, chlorophyll *a* and *b*, C and N content,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the leaf samples of a common herbaceous plant *Taraxacum officinalis* were measured. The stomatal pore surface and minimal stomatal resistance of *T. officinalis* varied significantly between land use classes. In the harbor and industry land use class and the urban land use class a 27% and 21% lower mean stomatal pore surface at the abaxial leaf surface, and a 29% and 27% lower mean stomatal pore surface at the adaxial leaf surface was observed compared to that in the pasture land use class. The minimal stomatal resistance at the abaxial leaf surface was significantly higher in the urban land use class and harbor and industry land use class by 28% and 29%, respectively compared to that in the pasture land use class. In addition, urbanized and industrial land use classes as the harbour and industry and the urban land use classes showed significantly lower  $\delta^{13}\text{C}$  values compared to pasture land use class. The specific leaf area, stomatal parameters and  $\delta^{13}\text{C}$  data were geostatistically analyzed to understand their spatial variation. The spatial distributions of stomatal pore surface and minimal stomatal resistance varied considerably across the study area, indicating a different habitat quality from the harbour area in the north, over the city centre in the middle and the industrial areas in the south, compared to off city areas. Spatial patterns of  $\delta^{13}\text{C}$  showed depleted  $\delta^{13}\text{C}$  levels in city areas indicating the diluted  $\delta^{13}\text{C}$  in the urban atmosphere by fuel combustion. We concluded that stomatal characteristics seem to be the most promising parameter for estimating urban habitat quality.

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### 1. Introduction

Urban ecosystems are comprised of diverse land uses including commercial, industrial, residential, transport, recreational, agricultural and nature areas, resulting in different habitats for plants, animals and humans within the urban landscape. Urban habitat quality comprises the integration of different abiotic and biotic components making up the habitat, such as air, soil and water quality, microclimate and the presence of vegetation. Due to variations in both the abiotic and biotic components urban habitat quality will vary between different land uses.

Data on atmospheric, soil or water quality are erroneous or inadequate to show variations of urban habitat quality since these data do not provide information on synergistic, antagonistic or additive effects of chemicals in complex pollutant mixtures and integrated responses in biological tissues which vary with prevailing microclimatic conditions. Biological monitoring procedures are thus required to evaluate urban habitat quality. Advantages of biomonitoring have been discussed frequently (Martin and Coughtrey, 1982; Wittig, 1993).

Lichens are the most studied species as biomonitors of urban air quality. In numerous studies their bioaccumulation potential of trace elements and alteration of physiology and population changes due to poor air quality have been proven (Bennett and Wetmore, 1999; Ra et al., 2005). Tree species such as oak (*Quercus ilex*) (Alfani et al., 2000), and pine needles (Jensen et al., 1992) also showed significant potential for accumulating trace elements and persistent

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organic compounds. *Taraxacum officinalis* is a common herbaceous weed that has been frequently studied as a biomonitor of environmental pollution (Kuleff and Djingova, 1984; Djingova and Kuleff, 1999) and has been used in a number of regional scale studies in Poland (Kabata-Pendias and Dudka, 1991), Hungary (Kovac et al., 1993), USA (Keane et al., 2001), Germany (Winter et al., 1999), and Canada (Marr et al., 1999). Most of these studies focused, however, on the use of *T. officinalis* for biomonitoring of trace metals.

Plant physiological parameters have also been used frequently as bio-indicators of urban habitat quality. For example, chlorophyll fluorescence (Sgardelis et al., 1994), chlorophyll content (Ali, 1991), photosynthetic gas exchange rate, stomatal conductance and transpiration rate (Moraes et al., 2003) showed significant responses to air quality. Also analysis of visible leaf damage together with leaf chemical contents in various standard species (*N. tabacum* Bel W3, *Petunia hybrida* cv. Mirage, *Gladiolus hybrida* cv. White friendship, *L. multiflorum italicum* cv. Lema) have been used to characterize local air pollution (Klumpp et al., 1994; Moraes et al., 2002).

Less attention has been given to morphological and anatomical parameters of plants as indicators of long-term responses to changing (urban) habitat quality, although parameters as specific leaf area, stomatal density and pore surface were recognized to vary depending on microclimatic conditions (Barber et al., 2004). Moreover, sampling and analysis of these parameters is relatively easy and inexpensive.

Beside the limited attention given to morphological and anatomical plant parameters, the use of stable isotope techniques in plant ecological research has grown steadily during the past two decades (Dawson et al., 2002). Stable isotopes can serve as integrators of how plants interact with, and responded to, the quality of their habitat. Genetic and environmental factors that influence gas exchange through morphological and functional plant responses cause changes in plant  $\delta^{13}\text{C}$  content (Ehleringer, 1993). For example,  $\delta^{13}\text{C}$  in plants were found to reflect the habitat quality in relation to the land use in Karlsruhe (Germany) and Gent (Belgium) (Norra et al., 2005; Boeckx et al., 2006).  $\delta^{13}\text{C}$  in ambient air is about  $-8\%$ . Fossil fuel burning can locally reduce this value to about  $-12\%$  (Kiyoshu and Kidoguchi, 1998). Therefore,  $\delta^{13}\text{C}$  content of plants grown in areas with poor air quality due to fossil fuel burning is expected to be lower compared to areas with a better air quality. In addition, variation in foliar  $\delta^{13}\text{C}$  content has been related to leaf size (Geber and Dawson, 1990) and thickness and stomatal density (Hultine and Marshall, 2000).

The main objective of this study was to assess the potential of anatomical leaf characteristics (specific leaf area, stomatal density and dimension) and leaf (bio)chemical characteristics (chlorophyll *a* and *b*, C and N content,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) as effective parameters for biomonitoring of urban habitat quality.

## 2. Materials and methods

### 2.1. Study area description

The study area covered 81.5 km<sup>2</sup> within the urban and suburban surrounding of the city of Gent, Belgium (51°00'N, 3°50'E). Gent urban area includes several industrial zones, densely populated areas, a harbour and network of busy highways and train tracks, urban green, agriculture and nature areas (Boeckx et al., 2006). The predominant wind flow across the area is from southwest to northeast. Besides the densely populated central area several moderately densely populated areas are situated at northwest (Wondelgem–Mariakerke) northeast (Oostakker–Sint–Amandsberg and Destelbergen) and southeast (Heusden, Melle–Merelbeke and Ledebeg–Gentbrugge) of the study area (Fig. 1). In the north of the study area, the harbour of Gent and associated industrial areas

are situated. Main industrial, commercial and service areas are situated around the city centre and in the southern part of the city near the crossing of E17 and E40 European highways. The agricultural area consists of pasture and arable land and is mainly situated in the eastern and upper northwest part of the study area. The Bourgoyen–Ossemeersen nature reserve which mainly consists of wet meadows covers a large area at the eastern part (220 ha). Forested areas are rather limited and scattered. Urban green areas are even more scattered.

The different land uses of the study area were classified into 4 land use classes: urban (U) including the city centre and suburban areas and roads, urban green (UG) including urban green and forested areas, harbour and industry (HI) including the harbour, industrial areas and railways, and pasture (P) land use class covering all pasture and grass lands (Fig. 1).

### 2.2. Plant sampling

Fig. 2 shows the position of the 148 sampling locations inside the study area. These georeferenced sampling locations were obtained from a previous study (Boeckx et al., 2006), and were selected within the different land use classes as mentioned above, using a partially random and partially grid sampling design. Dandelion (*T. officinalis* Weber complex), a commonly distributed wild herb species, was selected for the study. At each sampling location, four fully grown plants at flowering stage which were 1–3 m apart were selected for sampling from mid August till mid October 2005.

### 2.3. Specific leaf area and stomatal characteristics

From each of the four selected plants at each sampling location, four fully grown undamaged leaves were collected. Specific leaf area (SLA) (cm<sup>2</sup> g<sup>-1</sup>) of each plant was calculated by dividing total single sided fresh leaf area (measured with Li-3000 leaf area meter, Li-COR, Lincoln, Nebraska) by its oven dry weight (60 °C for 24 h).

Stomatal imprints at both the adaxial and abaxial side of a fully grown mature leaf of each sampled plant were collected to determine stomatal density (SD) (the number of stomata per mm<sup>2</sup> leaf area) and stomatal pore surface (SPS) (the surface area of a widely opened stomatal pore, μm<sup>2</sup>). Stomatal imprints were made on attached leaves by applying colourless nail varnish and peeling off the surface with a transparent adhesive tape which was then fixed on a microscope slide. Nail varnish was applied to an area of approximately 1 cm<sup>2</sup> in the middle portion of the selected leaves (Elagöz et al., 2006), to the right of the mid vein, avoiding areas overlying veins. Stomatal imprints were analyzed with a light microscope (Wild Leitz GMBH 020-505.030) connected with a TV screen at a magnification of 40 × 10. First, the statistical minimal number of microscopic fields ( $N_{\min}$ ) that should be counted on a single imprint was calculated for each land use class using the Student-*t*-test (Eq. (1)). Therefore, in four randomly selected sub-sampling points of each considered land use class the number of stomata in 25 microscopic fields was counted to determine  $N_{\min}$ .

$$N_{\min} = \left[ \frac{2.064^2}{\bar{X}^2/100} \right] \times S^2 \quad (1)$$

where 2.064 is the *t*-value for (25-1) degrees of freedom, with a threshold value of *p* = 0.05, *X* and *S* are the mean and the standard deviation of the counted stomata in the microscopic fields, respectively. Subsequently, the number of stomata for each stomatal imprint was counted on  $N_{\min}$  microscopic fields for the respective land use class. SD was calculated by dividing the amount of stomata by the surface area of a microscopic field after calibration with a calibration plate. The SPS (μm<sup>2</sup>) was calculated by measuring length (*L*) (m) and width (*W*) (m) of widely opened stomata,

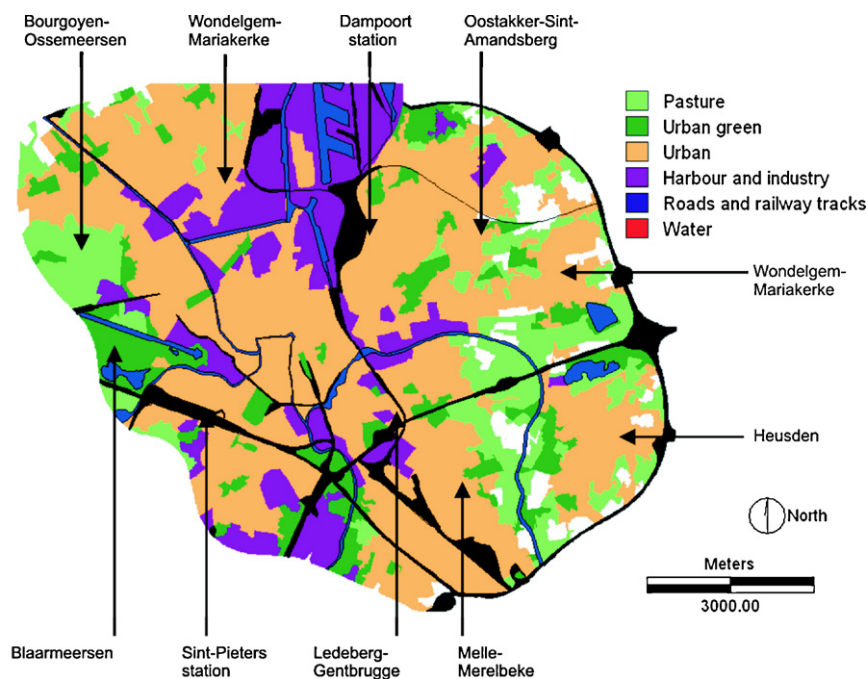


Fig. 1. Study area with indication of the 4 land use classes (map scale is in m according to the Belgian National Lambert72 projection).

and by assuming an elliptical shape:

$$SPS = \frac{L \times W \times \pi}{4} \quad (2)$$

The theoretical minimal stomatal resistance ( $R_s$ ) was calculated as:

$$R_s = \frac{4l}{n\pi LWD} + \frac{L+W}{4nLWD} \quad (3)$$

where  $R_s$  is the stomatal resistance ( $s\ m^{-1}$ ),  $l$  is the stomatal pore depth (m),  $D$  is the diffusion coefficient of water vapour in air ( $24.2 \times 10^{-6}\ m^2\ s^{-1}$  at  $20^\circ C$ ) and  $n$  is the stomatal density (number of stomata  $m^{-2}$ ). Stomatal depth was assumed as  $10\ \mu m$  (Samson et al., 2000; Olyslaegers et al., 2002).

#### 2.4. Sample preparation and chemical analysis

A composite sample for chemical analysis was made of the dried leaves from the four sub-samples collected at each location. The pooled samples could be considered as point samples relative to the size of the investigated area. Sub-samples were ground using an ultra-centrifugal mill (ZM200, Retsch, Germany). Samples were weighed separately in tin cups and analyzed in triplicate for C (%), N (%),  $\delta^{13}C$  (‰) and  $\delta^{15}N$  (‰) using an elemental analyzer (EA) (ANCA-SL, Europa PDZ, UK) coupled to an Isotope Ratio Mass Spectrometer (IRMS) (20-20, SerCon, UK) (Boeckx et al., 2006). Isotope ratios and percent element were measured during one sample run, i.e. the IRMS was tuned in “dual mode” to enable simultaneous analysis of C and N. Sub-samples were also used for the spectrophotometric (Uvikon 930, Kontron Instruments, Milan, Italy) determination

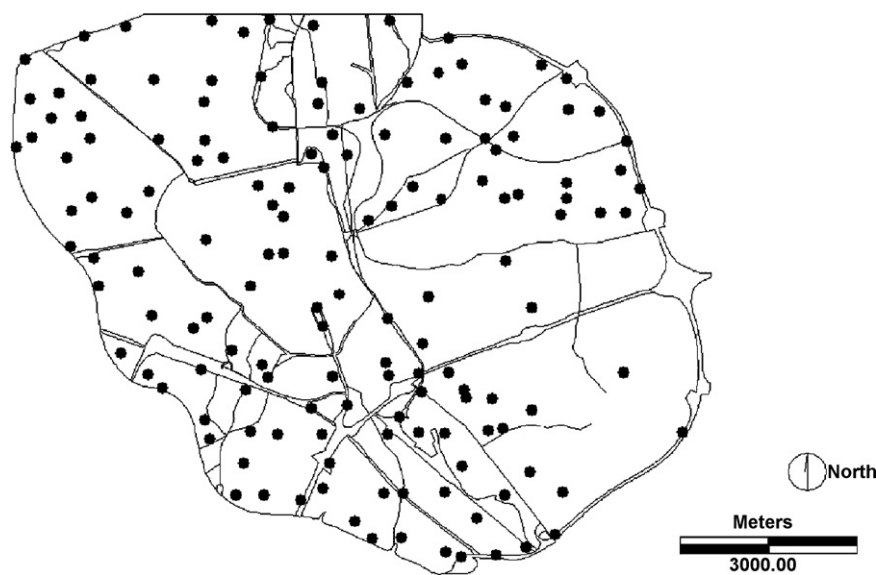


Fig. 2. Location of the 148 sampling locations (dots) in the study area.

of chlorophyll *a* and *b* ( $\text{g mg}^{-1}$ ), according to the methodology described by Lichtenthaler (1987).

### 2.5. Data analysis and geostatistical data processing

First, the temporal effect of the sampling duration was examined by means of linear regression between the measured variables and the date of sampling, but no effect was observed. Differences of the plant parameters between the land use classes were analyzed using analysis of variance (ANOVA) with Duncan test ( $p = 0.05$ ) using SPSS 12.0 software.

Geostatistical analysis was carried out for several selected parameters (SLA, abaxial SPS, abaxial  $R_s$ , and  $\delta^{13}\text{C}$ ). Spatial distribution of the parameters which did not show differences across land use classes was elucidated by means of ordinary kriging. Simple Kriging with Varying Local Means (SKLM) method was applied to the parameters which show significant differences between land use classes. Both methods are based on the general kriging estimator with different assumptions. Consider a random variable  $Z$  that has been measured at  $n$  locations,  $z(\mathbf{x}_\alpha)$ ,  $\alpha = 1, \dots, n$ , the kriging estimator at an unsampled location  $\mathbf{x}_0$  can be written as:

$$Z^*(\mathbf{x}_0) - m(\mathbf{x}_0) = \sum_{\alpha=1}^{n(\mathbf{x}_0)} \lambda_\alpha \cdot [Z(\mathbf{x}_\alpha) - m(\mathbf{x}_\alpha)] \quad (4)$$

where  $n(\mathbf{x}_0)$  is the number of neighbourhood measurements  $z(\mathbf{x}_\alpha)$  used for estimating  $Z^*(\mathbf{x}_0)$ ,  $\lambda_\alpha$  are the weights assigned to data  $z(\mathbf{x}_\alpha)$  which are considered to be a realization of the random variable  $Z$  and  $m(\mathbf{x}_0)$  and  $m(\mathbf{x}_\alpha)$  are the expected values (or means) of  $Z^*(\mathbf{x}_0)$  and  $Z(\mathbf{x}_\alpha)$ . In ordinary kriging, it is assumed that of  $Z^*(\mathbf{x}_0)$  and  $Z(\mathbf{x}_\alpha)$  are unknown, but locally stationary. The SKLM procedure uses the secondary information, in this case land use class, to estimate  $Z^*(\mathbf{x}_0)$  and  $Z(\mathbf{x}_\alpha)$ . Extensive discussion on these kriging methods can be found in Goovaerts (1997).

## 3. Results

### 3.1. Variation between land use classes

The SLA varied between 130.1 and 472.1  $\text{cm}^2 \text{g}^{-1}$  over the study area (Table 1). Although SLA was not significantly different between land use classes, mean SLA in UG, HI, and U was 297.8, 283.1, 273.8  $\text{cm}^2 \text{g}^{-1}$ , respectively, which was an increase by 22%, 16% and 12% compared to the mean SLA (244.8  $\text{cm}^2 \text{g}^{-1}$ ) in P.

Abaxial SPS varied between 11.0 and 44.5  $\mu\text{m}^2$  over the study area, while adaxial SPS ranged from 15.5 to 64.6  $\mu\text{m}^2$  (Table 1). As illustrated in Fig. 3a, mean abaxial SPS in HI and U were significantly lower compared to that of UG and P. This decrease in HI and U was 27% and 21%, respectively, compared to P. Similar differences between land use classes were observed for adaxial SPS (Fig. 3b). Stomatal density at the abaxial side varied from 136.8 to 314.1 stomata  $\text{mm}^{-2}$  over the study area. At the adaxial side, SD was lower than the abaxial values and ranged from 56.8 to 173.0 stomata  $\text{mm}^{-2}$  of leaf area (Table 1). SD showed an opposite pattern of variation between land use classes compared to that observed for SPS. The adaxial variation in SD was more pronounced compared to the abaxial variation, with a mean adaxial SD in HI of 121.2  $\text{mm}^{-2}$  which was 13% higher than SD in P (107.4  $\text{mm}^{-2}$ ).  $R_s$  at the abaxial side varied from 41.8 to 250.3  $\text{s m}^{-1}$ . At the adaxial side  $R_s$  was higher and ranged from 66.5 to 303.9  $\text{s m}^{-1}$  (Table 1). Both abaxial and adaxial  $R_s$  was higher in HI and U than in P and UG. For both sides, mean  $R_s$  in U was significantly ( $p < 0.05$ ) different from P and UG, while for HI this was only the case for the abaxial side.

Chlorophyll *a* content varied between 30.8 and 158.4  $\text{mg g}^{-1}$  within the study area, whereas, chlorophyll *b* content varied from 15.2 to 127.3  $\text{mg g}^{-1}$  (Table 2). Carbon content ranged from 35.0% to

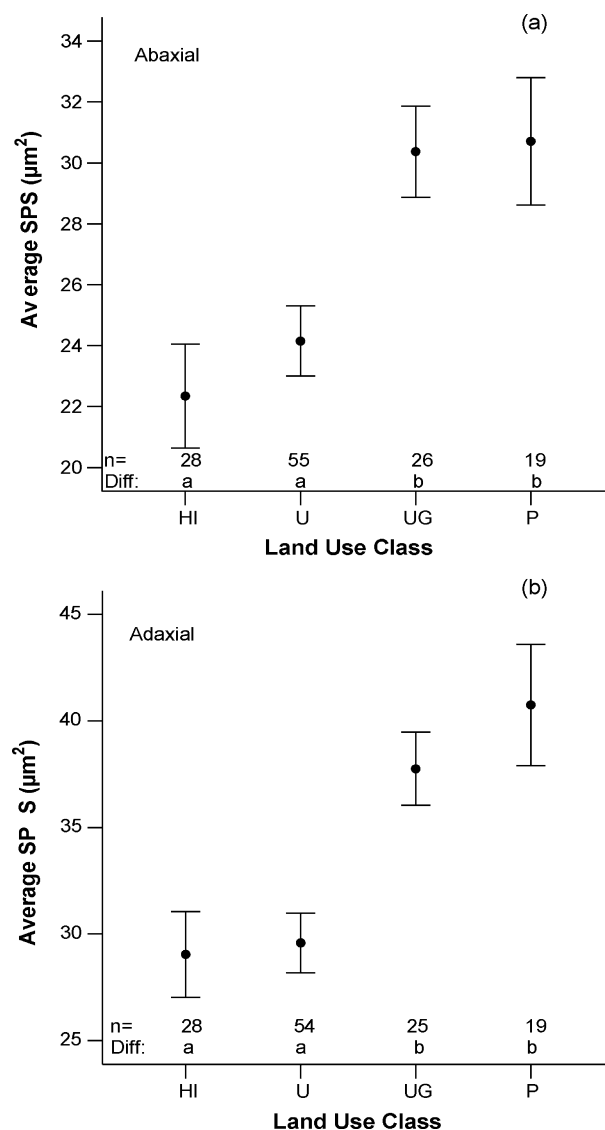


Fig. 3. Abaxial (a) and adaxial (b) average stomatal pore surface (SPS) ( $\mu\text{m}^2$ ) of *T. officinalis* in four land use classes: P=pasture, UG=urban green (urban green plus forest areas), U=urban (city, suburban plus road areas), HI=Harbor and Industry (harbor, railways plus industrial areas); error bars represent  $\pm 1$  standard deviation;  $n$ =number of observations per class; different letters indicate significantly different mean values at  $\alpha = 0.05$  (Duncan test).

56.2%, while N content of these leaves ranged from 0.9% to 6.3%.  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values ranged from  $-4.7\text{‰}$  to  $6.9\text{‰}$  and from  $-34.3\text{‰}$  to  $-26.3\text{‰}$ , respectively. Among the analyzed leaf (bio)chemical parameters chlorophyll *b*, *c* and N content were not significantly different between the land use classes. However, chlorophyll *a* content in UG was significantly higher compared to P and HI land use classes, for which the lowest values were observed. Stable isotope abundances were not significantly different between land use classes for  $\delta^{15}\text{N}$ , while significantly higher  $\delta^{13}\text{C}$  values were observed in P compared to the other land use classes. The urbanized and industrial land use classes (U and HI) showed the lowest  $\delta^{13}\text{C}$  values.

### 3.2. Spatial distribution

SLA was ordinary krigged to understand its spatial variation. However, significant differences observed for abaxial SPS, abaxial  $R_s$  and  $\delta^{13}\text{C}$  across different land use classes allowed to consider land use as secondary information to predict these variables using SKLM. Firstly, the experimental omnidirectional variograms of SLA

**Table 1**  
Descriptive statistics of specific leaf area (SLA), stomatal pore surface (SPS), stomatal density (SD), stomatal resistance ( $R_s$ ) of *T. officinalis* in the study area ( $n = 146$ ). Different letters indicate significantly different mean values between the abaxial and adaxial side ( $p < 0.05$ ).

	SLA ( $\text{cm}^2 \text{g}^{-1}$ )		SPS ( $\mu\text{m}^2$ )		SD (no. $\text{mm}^{-2}$ )		$R_s$ ( $\text{s m}^{-1}$ )	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Min	130.1	11.0	15.5	136.8	56.8	41.8	66.5	
Max	472.1	44.5	64.6	314.1	173.0	250.3	303.9	
Mean	277.3	26.0 <sup>a</sup>	32.8 <sup>b</sup>	214.5 <sup>a</sup>	117.2 <sup>b</sup>	110.0 <sup>a</sup>	158.0 <sup>b</sup>	
Median	271.8	25.2	33.0	215.1	117.0	97.6	144.4	
StD	67.0	9.1	11.3	33.7	23.2	43.3	52.2	
$g_1$	0.48	0.069 <sup>*</sup>	0.36 <sup>*</sup>	0.032 <sup>*</sup>	0.10 <sup>*</sup>	1.13	0.84	
$g_2$	0.37 <sup>*</sup>	-1.14	-0.66 <sup>*</sup>	-0.30 <sup>*</sup>	-0.33 <sup>*</sup>	0.80	0.21 <sup>*</sup>	

Min = minimum value, Max = maximum value, StD = standard deviation,  $g_1$  = skewness,  $g_2$  = kurtosis.

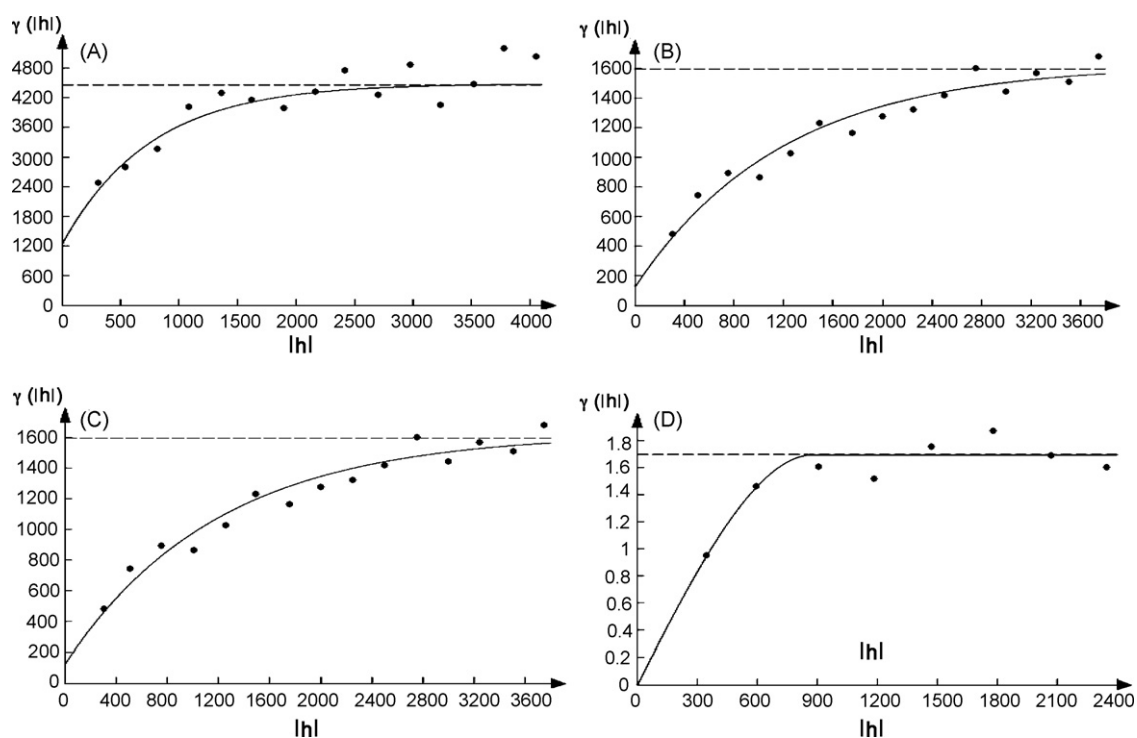
<sup>\*</sup> Significant normal distribution by  $t$ -test at  $\alpha = 0.05$ .

**Table 2**  
Descriptive statistics of chlorophyll *a* and chlorophyll *b*, C and N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *T. officinalis* in the study area ( $n = 146$ ).

	Chlorophyll <i>a</i> ( $\text{mg g}^{-1}$ )	Chlorophyll <i>b</i> ( $\text{mg g}^{-1}$ )	C (%)	N (%)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Min	30.8	15.2	35.0	0.9	-34.3	-4.7
Max	158.4	127.3	56.2	6.3	-26.3	6.9
Mean	108.3	58.2	47.7	3.4	-30.2	1.8
Median	112.6	56.7	40.7	3.2	-30.3	1.9
StD	30.8	24.7	4.4	1.1	1.4	5.9
$g_1$	-0.58	0.41 <sup>*</sup>	0.86	0.26 <sup>*</sup>	0.3 <sup>*</sup>	-0.3 <sup>*</sup>
$g_2$	-0.57	0.26 <sup>*</sup>	0.22 <sup>*</sup>	-0.21 <sup>*</sup>	0.5 <sup>*</sup>	-0.2 <sup>*</sup>

Min = minimum value, Max = maximum value, StD = standard deviation,  $g_1$  = skewness,  $g_2$  = kurtosis.

<sup>\*</sup> Significant normal distribution by  $t$ -test at  $\alpha = 0.05$ .



**Fig. 4.** Omnidirectional experimental variograms (dots) and fitted models (line) of SLA (a), abaxial SPS (b), abaxial  $R_s$  (c) and  $\delta^{13}\text{C}$  (d),  $|h|$  = lag distance in m. Note the different scales of the Y axis due to  $\gamma(|h|)$  = semivariance in square units of the measurement units.

**Table 3**  
Omnidirectional variogram model parameters of SLA, SPS,  $R_s$  and  $\delta^{13}\text{C}$ , with indication of the used model, the range, sill, nugget and the relative nugget effect.

Variable	Model	Range (m)	Sill <sup>a</sup>	Nugget <sup>a</sup>	Rel. nugget effect (%)
SLA ( $\text{cm}^2 \text{g}^{-1}$ )	Exponential	2255	4500	1260	28
Abaxial SPS ( $\mu\text{m}^2$ )	Exponential	4000	66	14	22
Abaxial $R_s$ ( $\text{s m}^{-1}$ )	Exponential	3572	1629	128	8
$\delta^{13}\text{C}$ (‰)	Spherical	881	1.7	0	0

<sup>a</sup> The units are equal to the square of the variable units.

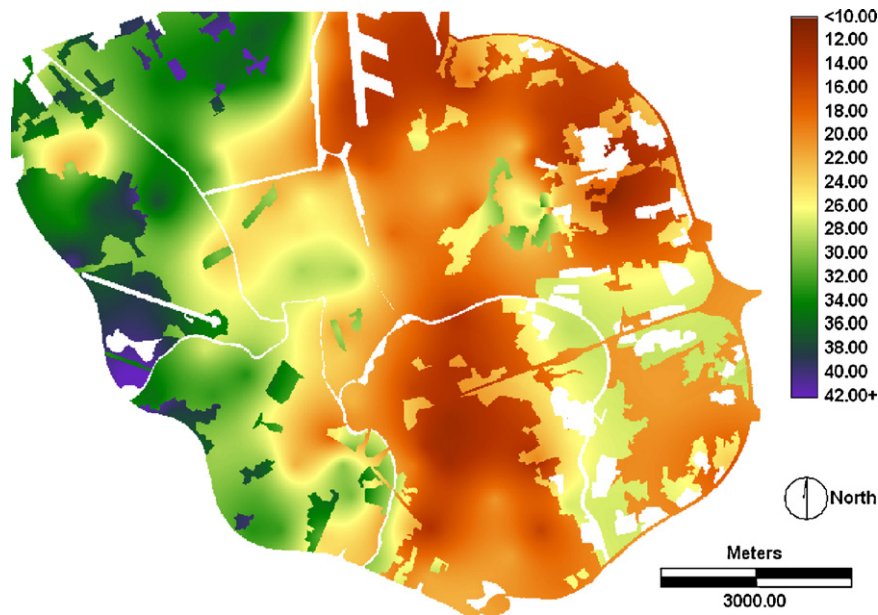


Fig. 5. Spatial distribution of abaxial stomatal pore surface (SPS) in the study area obtained by SKLM.

and of the residuals of abaxial SPS, abaxial  $R_s$  and  $\delta^{13}\text{C}$  were calculated and modelled using Variowin software (Fig. 4). Table 3 summarises the variogram parameters; range, sill, nugget and relative nugget effect of the modelled variograms. The maximum value of semivariance ( $\gamma$ ) is known as the sill. The lag distance ( $h$ ) where variogram reaches to sill is known as range, which is the critical part of variogram because the observations located within the range are spatially correlated. The semivariance when  $h=0$  is known as nugget, which is an estimate of the residual error or spatially uncorrelated noise. Relative nugget effect is the ratio between nugget and sill (Burrough and McDonnell, 1998), which quantifies the strength of spatial structure. All variograms of the considered parameters show a bounded structure with ranges of 881–4000 m. The small relative nugget effects (0–28%) depicted by all parameters indicated a strong spatial correlation inside their ranges (Goovaerts, 1997).

It is clear that in the central part of the study area, SPS decreased from the industrial zone and Melle–Merelbeke resi-

dential area (southern part), over the city centre (middle) to the harbour in the northern part (Fig. 5). In addition, lower values were observed in the residential areas in Oostakker–Sint–Amandsberg and Destelbergen suburban areas in north east and Heusden suburban area in the eastern part. High SPS was observed in western part of the study area in Bourgoyen–Ossemeersen nature reserve, in Blaarmeersen recreational area and the surrounding areas with high biological value, and also in Wondelgem–Mariakerke suburban area which consist of residential areas with gardens. Intermediate values were found in pasture areas in the east and south-eastern part of the study area. Two exceptional distributions could be seen with high SPS values in the southwest of the study area around Gent Sint-Pieters railway station and intermediate values in the southern industrial area. In contrast to the SLA map (not shown) all pasture areas, urban green and forested areas are prominently appearing in the SPS map (Fig. 5).

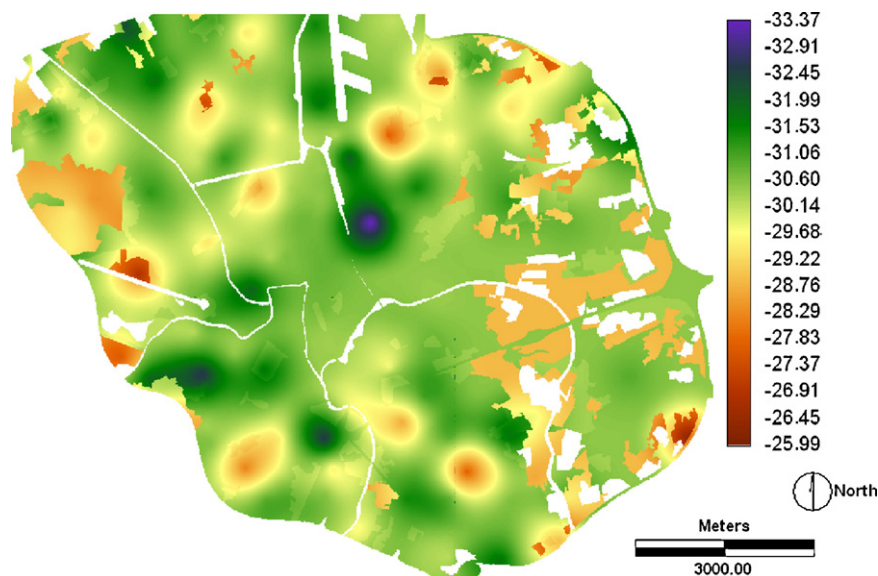


Fig. 6. The spatial distribution of  $\delta^{13}\text{C}$  (‰) in the study area obtained by SKLM.

Highest  $\delta^{13}\text{C}$  was observed around Dampoort and Sint-Pieters railway stations, which are also busy traffic points, and at Ledeburg where the E17 highway enters the city (Fig. 6). In addition, higher values were observed around the harbour, in the city centre, in the suburban residential areas in Oostakker–Sint–Amandsberg and Destelbergen in the northeast, Heusden suburban area in the eastern part and Melle–Merelbeke residential area at the southern part. Low values were observed in grasslands including the Bourgoyen–Ossemeersen nature reserve, Blaarmeersen recreational area, and pasture areas in the eastern part.

The spatial distribution of  $R_s$  (map not shown) yielded mostly comparable information to that of SPS (Fig. 5), whereas the spatial distribution of SLA (map not shown) did not allow distinguishing clear spatial patterns which could be easily linked to land use (see also Table 3).

## 4. Discussion

### 4.1. Spatial distribution of SLA

Leaf area and specific leaf area have been studied at several locations as a biomonitoring parameter of urban habitat quality, especially urban air quality (Carreras et al., 1996; Lima et al., 2000; Moraes et al., 2002; Verma and Singh, 2006).

SLA of *T. officinalis* measured by Poorter and De Jong (1999),  $368\text{--}378\text{ cm}^2\text{ g}^{-1}$ , was higher than our mean value observed, but fell within the observed range in this study (Table 1). We observed significantly higher SLA values in UG and HI which are located in more urbanized and intensive road traffic areas compared to P located in less urbanized areas (Fig. 1). The spatial distribution map of SLA (map not shown) tends to indicate high values in dense traffic and industrialised areas and low SLA values in the less urbanised areas including the nature reserve Bourgoyen–Ossemeersen. In agreement with our results, Carreras et al. (1996) found a significant increase of SLA by 38% in *Ligustrum lucidum* in areas with high road traffic compared to less intensive road traffic areas in the city of Córdoba, Argentina.

Several studies found a leaf area reduction in polluted sites in comparison to less polluted sites. For example, a 40% and 17% leaf area reduction was observed in polluted sites in comparison to less polluted sites in India for two tree species namely, *Ficus religiosa* and *Thevetia nerifolia*, respectively (Verma and Singh, 2006). Lima et al. (2000) and Moraes et al. (2002) also observed a reduction in leaf area of *Phaseolus vulgaris* and *Tibouchina pulchra* in polluted city areas of Brazil compared to forested areas. In agreement we observed a leaf area reduction in more urbanized land use classes, i.e. 37% and 39% reduction in U and UG compared to P, respectively. As SLA increased in more industrialised land use classes compared to P (data and map not shown), and simultaneously leaf area decreased (data not shown), leaf thickness should have been seriously reduced in the more urbanized areas suggesting plants invested less energy in their leaves as their longevity decreased due to poor habitat quality. Smaller leaves reduce the contact area between plant and atmosphere, which leads to a reduced absorption of obnoxious gases and a decreased deposition on the leaf's surface. Decreased urban habitat quality to an inferior air quality can thus largely explain the observed differences in our study area. However, the largest mean SLA is observed in UG compared to HI, U and P. This could be an adaptation to possible lower irradiance levels in shaded habitats (Ashton et al., 1999) in UG where trees and shrubs are abundant. In contrast, *T. officinalis* is more a species of open terrains. Frequent mowing in home gardens in Wondelgem–Mariakerke in the northwest corner of the study area, may be another reason for thick leaves, and thus low SLA values. Unexpected low SLA values observed in a larger part of the harbour

area cannot be related to road traffic or shaded conditions in this habitat. However, soil or water availability can be stress factors in this area.

### 4.2. Spatial distribution of stomatal characteristics

In accordance with our results, *T. nerifolia* showed a 47% increase in SD and 30% decrease in stomatal size at polluted sites compared to a less polluted site in India (Verma and Singh, 2006). However, in the same study the opposite result was obtained for *F. religiosa*, with a 59% reduction in SD and a 40% increase in stomatal size at the high polluted site. Garg and Varshney (1980) also reported a reduction of stomatal size by air pollution. Alessio et al. (2002) observed an increase in stomatal resistance in response to air pollution by road traffic. Stomatal responses are regarded as avoidance mechanisms plants employ in controlling the amount of air pollutants (e.g.  $\text{O}_3$ ) entering the leaves (Andersen, 2003). As suggested by Poole et al. (2000), both SD and aperture (here indicated by SPS) affect stomatal resistance, as they are both inversely proportional to  $R_s$  (see Eq. (3)). We observed a spatial distribution of abaxial SPS with low values in a north south central band in the study area, where more urbanization and road traffic is concentrated (Fig. 5), which could indicate an increased stomatal resistance in these areas. However, the higher SD observed in these areas suggests a reduced stomatal resistance. The net effect is that the calculated abaxial stomatal resistance increased along a more or less similar north south central band in the study area (map not shown) indicating decreased stomatal size overshadows increased stomatal density. So, our measurements clearly indicate plants react to a decreased habitat quality, mainly due to air pollution, by decreasing the size of the stomata and increasing their density. This way plants can more 'fine-tune' their gas exchange with the environment, thereby avoiding as much as possible the uptake of air pollutants, and optimising  $\text{CO}_2$ -uptake for photosynthesis in relation to water losses by transpiration.

### 4.3. Spatial distribution of $\delta^{13}\text{C}$

We observed significantly higher  $\delta^{13}\text{C}$  values (less negative) in P compared to the other land use classes. More urbanized and industrial land use classes (HI and U) showed lowest  $\delta^{13}\text{C}$  values (more negative). The spatial distribution of  $\delta^{13}\text{C}$  indicates highest  $\delta^{13}\text{C}$  values around Dampoort and Sint-Pieters railway stations and at Ledeburg where the E17 highway enters the city (Fig. 6). At all these points the intensive traffic is slowing down. In addition, higher values were observed around the northern harbour, in the city centre and several suburban residential areas compared to low values in eastern grasslands and Bourgoyen–Ossemeersen. This depletion in foliar  $\delta^{13}\text{C}$  values can be due to the decreased average  $\delta^{13}\text{C}$  of ambient air in areas with high road traffic and domestic heating. Theoretically, plants growing in fossil–fuel– $\text{CO}_2$ -contaminated areas, such as major cities, industrial centres, and highway borders, should assimilate a mixture of atmospheric  $\text{CO}_2$  with a  $\delta^{13}\text{C}$  value of  $-8.0\%$  and of fossil fuel derived  $\text{CO}_2$  with a  $\delta^{13}\text{C}$  value of  $-27.3\%$ . This isotopic difference should, thus, be recorded in plant carbon. Lichtfouse et al. (2003) revealed that grasses growing near a major highway in Paris (France) have strikingly depleted  $\delta^{13}\text{C}$  values, averaging at  $-35.1\%$ , versus rural grasses that show an average  $\delta^{13}\text{C}$  value of  $-30.6\%$ . We observed a lower average depletion (about 2%) in  $\delta^{13}\text{C}$  in *T. officinalis* in the urban area of Gent (about 230,000 inhabitants) which can be related to a lower degree of urbanization and traffic than in Paris and a lower sensitivity of the selected plant species. As our study was conducted in the same study area as used by Boeckx et al. (2006) and was mainly based on the same sampling points, we can assume a mean soil  $\delta^{13}\text{C}$  of  $-27.2\%$  as reported by Boeckx et al. (2006). Compared to this mean  $\delta^{13}\text{C}$  value,  $\delta^{13}\text{C}$  of



*T. officinalis* was 3.0‰ depleted. Boeckx et al. (2006) and Norra et al. (2005) found a relative depletion for grass, in more urbanized areas compared to pasture land in Gent, and not specified herbs in Karlsruhe (Germany) of 2.7‰ and 3.9‰, respectively. Boeckx et al. (2006) attributed this to increased fossil fuel burning and the proximity to road traffic. However, at the three areas, where maximum  $\delta^{13}\text{C}$  values were observed in this study (Dampoort and Sint-Pieters railway stations and at the entrance of E17 highway in Lederberg where traffic slows down), average  $\delta^{13}\text{C}$  in *T. officinalis* was  $-33.8\text{‰}$ , showing a 5‰ depletion, which is in agreement with the study by Lichtfouse et al. (2003). Air samples were collected at these 3 places to check the expected depletion of  $\delta^{13}\text{C}$  in ambient air. Results revealed an average  $\delta^{13}\text{C}$  of  $-11.0\text{‰}$  ( $-12.8\text{‰}$  to  $9.2\text{‰}$ ) in ambient air. In agreement with these results, fossil fuel burning decreased the average  $\delta^{13}\text{C}$  of ambient air from its atmospheric average ( $-8\text{‰}$ ) to values ranging from  $-13.5\text{‰}$  to  $-11.0\text{‰}$  in Toyama city (Japan) (Kiyoshu and Kidoguchi, 1998) and from  $-19.8\text{‰}$  to  $-8.8\text{‰}$  in Paris (France) (Widory and Javoy, 1998). However, air samples taken at Bourgoyen nature reserve also showed a  $\delta^{13}\text{C}$  value of  $-10.5\text{‰}$  ( $11.8\text{‰}$ – $9.15\text{‰}$ ). This can be due to recycling of respired  $\text{CO}_2$  by the predominantly C3 grass species in the area. Two opposing factors may influence ecosystem  $\delta^{13}\text{C}$  in urban areas: first, physiological stress caused by atmospheric and soil pollutants may reduce the ratio of intercellular to ambient  $\text{CO}_2$  and increase the expected values of  $\delta^{13}\text{C}$ , while second,  $\delta^{13}\text{C}$  dilution of the urban atmosphere by fossil fuel combustion may decrease  $\delta^{13}\text{C}$ , when plants incorporate the depleted air into their biomass (Flanagan et al., 2005). In contrast to our results, Norra et al. (2005) observed less negative plant  $\delta^{13}\text{C}$  downwind from the industrial area in the city of Karlsruhe (Germany) and suggested that the injurious effects of airborne pollution on stomatal aperture results in a reduced discrimination. Also the enriched  $\delta^{13}\text{C}$  values in grass samples observed by Boeckx et al. (2006) in Gent city centre were explained as reduced discrimination due to plant stress such as nutrient depletion, drought or air pollution.

#### 4.4. Potential as bio-indicator

Based on the variation between land use classes and the spatial distribution maps, stomatal characteristics, especially SPS and  $R_s$ , reveal the highest contrast between land use classes. The latter integrates changes in as well SD as size. Spatial distribution maps of stomatal characteristics allow to distinguish urban habitat quality with a very high spatial resolution, provided the sampling network is dense enough. As can be seen from Fig. 5 these parameters even allow to distinguish very clearly, e.g. the urban green areas. This result is due to the fact that stomata are the key factors in the vegetation–atmosphere interaction. Although, deposition of airborne particles will mainly occur at the adaxial side of the mainly horizontally oriented leaves of *T. officinalis*, a similar pattern of variation between land use classes of stomatal parameters at both sides showed that stomata at both surfaces react similarly to the environment. However, significantly higher SD and significantly lower SPS at the abaxial compared to the adaxial side suggests that abaxial sampling will be more effective. Also  $\delta^{13}\text{C}$  shows potential as bio-indicator. It reveals complementary information to stomatal characteristics, as can be seen from the isotopic signatures measured at both railway stations and some areas around Blaarmeersen recreational area, indicating relatively undisturbed wet meadows (Fig. 6). Results for SLA and chlorophyll *a* were less straightforward, as they can be influenced by a multitude of factors like water availability, nutrients, air temperature and light amongst others. Chlorophyll *b*, C, N and  $\delta^{15}\text{N}$  content of the *T. officinalis* leaves showed less potential as a bio-indicator since they were not significantly different between the land use classes in the study area.

## 5. Conclusions

The spatial distribution of  $\delta^{13}\text{C}$  of *T. officinalis* in Gent follows the urban land use and associated human activities. More depleted plant  $\delta^{13}\text{C}$  in more urbanized areas indicates contamination of the air with combustion products of fossil fuel. Spatial variation of anatomical parameters of *T. officinalis* as specific leaf area, stomatal pore surface and stomatal density indicate differences in urban habitat quality for plant growth. These plant parameters, especially those related to stomatal characteristics, have a large potential as easy to measure and cost-effective bio-indicators to monitor urban habitat quality. Specific leaf area is the easiest parameter to determine, but the stomatal parameters show the highest potential to discriminate between different land use classes. Just as reported by other authors we conclude that *T. officinalis* is a suitable bio-indicator of urban habitat quality as it is a commonly distributed species, which is easy to sample and shows a clear morphological and anatomical response to differences in habitat quality. Due to the fact that causes of variation in discrimination of  $\delta^{13}\text{C}$  are complex, correlations between  $\delta^{13}\text{C}$  and a single factor such as stomatal pore surface or stomatal density is problematic, and has not been observed in this study in contrast to what was observed by Hultine and Marshall (2000). Therefore, further studies are needed to establish such correlations, and to find out if other species, or other parameters, are more sensitive to differences in urban habitat quality and air pollution, and are thus better suited for passive biomonitoring.

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