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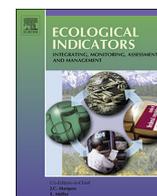
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## Original Articles

## Foliar optical traits indicate that sealed planting conditions negatively affect urban tree health



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## ABSTRACT

Urban trees play a key role in mitigating environmental problems in cities, but they often face harsh environmental conditions as they generally grow in sealed soils that have small rooting space and low water availability. In this context, rapid monitoring and assessment of tree health status is critical to maintain urban trees and secure the provisioning of urban ecosystem services. Across three European cities we selected 187 *Tilia tomentosa* trees growing under following planting conditions: (i) *sealed*, trees planted in small soil pits or strips surrounded by highly sealed surfaces (concrete, pavement or asphalt); and (ii) *unsealed*, trees planted in roomy soil surfaces (e.g. parks). We measured leaf reflectance and fluorescence and derived a set of optical traits from the measurements. We examined whether these non-destructively measured optical traits differ between planting conditions and whether they correlate with leaf functional traits, e.g. specific leaf area (SLA), leaf water content (LWC) and leaf water per area (LWA). Compared to the *unsealed* trees, *sealed* trees showed decreased SLA and LWC while increased LWA. Leaf optical traits differed between the *unsealed* and *sealed* trees. Highly sealed soils accelerated leaf senescence of the *sealed* trees compared to the *unsealed* trees, embodied in the temporal trend of optical traits. Sealed planting conditions negatively affect urban tree health status and phenology. These negative effects can be estimated by leaf optical traits, demonstrating the great potential of optical traits in assessing tree health status. Our findings provide insights into facilitating urban green management using optical traits and remote sensing data.

## 1. Introduction

Urban trees play key roles in providing ecosystem services and mitigating environmental problems in urban areas in the form of air quality improvement, microclimate regulation, noise reduction, moderation of the urban heat island effect and providing recreational and cultural values (Bolund and Hunhammar, 1999). Yet, urban trees generally grow in soils sealed by buildings and urban infrastructures and face harsh environmental conditions, including limited soil volume, soil

compaction and low soil moisture (Clark and Kjelgren, 1990; Sanders and Grabosky, 2014). Lack of rooting space, water and nutrient holding capacity will affect the development of urban trees, which leads to reduction in growth and health and imposing high risks of tree mortality (Grabosky and Bassuk, 1995). For instance, street trees planted in pits often have smaller canopies than trees planted in linear strips (Sanders and Grabosky, 2014). Moreover, urban trees are more prone to water deficits than forest trees, making them very susceptible to pathogens and pests (Clark and Kjelgren, 1990; Dale and Frank, 2017). All

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**Table 1**

Results of linear mixed models for testing the effect of different planting conditions (soil sealing conditions) on variations in leaf functional traits and optical traits. We set the two variables *City* and *Site* as random effect factors and used the Dataset 1 (Table S2) for the mixed models. Bold font highlights the statistical significance of each test ( $p < 0.05$ ).

Dependent variable	Model statistics		Post-hoc test (Tukey's HSD)					
	F-value	P-value	Boxed – Linear		Unlimited – Boxed		Unlimited – Linear	
			estimate	p-value	estimate	p-value	estimate	p-value
SLA	6.318	<b>0.002</b>	–0.605	0.535	4.235	<b>0.002</b>	3.63	<b>0.008</b>
LMA	7.773	<b>0.001</b>	0.315	0.383	–1.942	<b>&lt; 0.001</b>	–1.627	<b>0.004</b>
LWC	7.721	<b>0.001</b>	–1.399	<b>0.031</b>	4.156	<b>0.002</b>	2.757	0.051
LWA	3.923	<b>0.023</b>	–0.089	0.926	–1.208	<b>0.033</b>	–1.297	<b>0.019</b>
mSR705	5.708	<b>0.005</b>	–0.294	0.12	0.856	<b>0.008</b>	0.562	0.101
mND705	6.221	<b>0.004</b>	–0.029	0.062	0.072	<b>0.007</b>	0.043	0.141
NDWI	2.114	0.126	0.002	0.301	–0.004	0.188	–0.002	0.559
WI	1.067	0.349	–0.0002	0.934	0.002	0.318	0.002	0.409
MDWI	5.674	<b>0.004</b>	0.004	0.441	–0.026	<b>0.003</b>	–0.021	<b>0.017</b>
WI2	6.772	<b>0.002</b>	0.064	0.329	–0.345	<b>0.001</b>	–0.28	<b>0.01</b>
PRI	13.568	<b>&lt; 0.001</b>	–0.011	<b>0.009</b>	0.035	<b>&lt; 0.001</b>	0.023	<b>0.005</b>
PSRI	1.124	0.329	0.003	0.311	–0.001	0.983	0.002	0.734
SIPI	2.809	0.063	0.006	0.097	–0.007	0.185	–0.002	0.913
Fv/Fm	2.795	0.07	–0.007	0.222	0.015	0.106	0.008	0.488
PI	7.181	<b>0.001</b>	–0.257	0.612	2.103	<b>0.001</b>	1.845	<b>0.004</b>

this, in turn, will reduce the ecosystem services provided by trees in urban environments (Bolund and Hunhammar, 1999; Dale and Frank, 2017). In this context, monitoring urban tree health and, especially, water status is crucial to secure the provisioning of urban ecosystem services.

Water stress often leads to leaf morphological changes (Farooq et al., 2009; Fernández et al., 2002), such as reduced cell growth and small leaf area (Farooq et al., 2009). These morphological changes will induce variations in the observed functional traits such that a reduction in specific leaf area (SLA) and leaf water content (LWC) is often observed during water stress (Xu et al., 2009), which further affects whole plant growth (Farooq et al., 2009; Fernández et al., 2002). Therefore, the stress response is a complex of changes in traits that reflect plant strategies for coping with the stress (Farooq et al., 2009; Fernández et al., 2002; Wellstein et al., 2017). Water stress can also induce leaf senescence and alter tree phenology (Xie et al., 2015), disturbing ecosystem nutrient cycling and net primary production (Estiarte and Peñuelas, 2015). Therefore, quantitative determination of changes in leaf functional traits and changes related to leaf phenology during water stress is essential to understand plant strategies related to water stress.

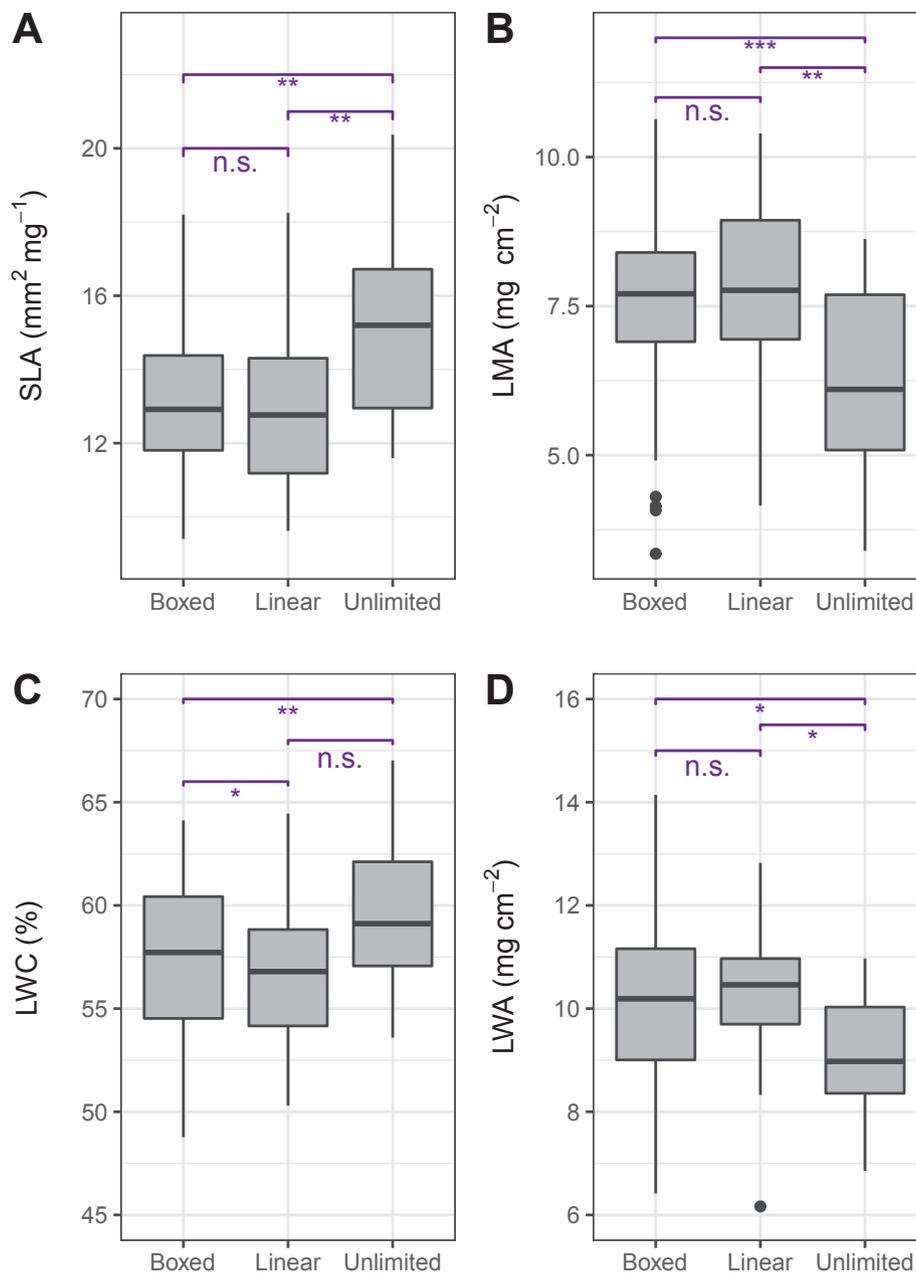
Efficient assessment tools for monitoring urban tree health status and measuring plant functional traits are still limited. Traditional approaches are mainly based on visual assessments and need additional effort to standardize the data protocol and to account for subjective bias in the data collected by different investigators (Roman et al., 2017). Visible symptoms of certain stresses, however, may take a long time to appear, whereas at the same time tree growth may have already been seriously inhibited (Smoleń, 2012). Thus, efficient monitoring approaches are needed so that one can estimate the stress before visible symptoms appear. Normally, stresses alter leaf biochemical characteristics before visible symptoms (Petrova et al., 2014; Smoleń, 2012). It is possible to detect stresses by measuring plant biochemical parameters (e.g., pigment, nutrient element) as stress indicators. However, these analyses are destructive, costly and time-consuming (de la Riva et al., 2016; Petrova et al., 2014), making continuous monitoring of tree health status infeasible.

Advances in optical sensing technologies provide a means to quantify optical properties of leaves and characterize their optical traits (Ollinger, 2011; Ustin et al., 2009). Leaf reflectance and fluorescence measurements both allow for a rapid extraction of leaf optical traits of interest related to plant health or vitality (Buschmann, 2007; Delalieux

et al., 2009; Gamon et al., 1992; Sims and Gamon, 2002). The former measures the passively reflected light energy whereas the later measures the actively emitted energy by leaves, and both are of great potential as alternatives of lab analyses for plant health assessment (Lichtenthaler et al., 1998). Reflectance- and fluorescence-based techniques are non-destructive and have great repeatability, which enables to monitor plant health *in situ* readily by analyzing plant optical traits (Gitelson et al., 2003; Lausch et al., 2016) and allows for remotely assessing ecosystem functions (Pettorelli et al., 2017).

A reflectance-based approach for monitoring plant health typically employs spectral indices (Rouse et al., 1974). Since decades, a diverse set of spectral indices has been developed and validated for the estimation of plant biological traits such as leaf pigments, area, water, nitrogen and photosynthesis (Gamon et al., 1992; Gao, 1996; Gitelson et al., 2003; Peñuelas et al., 1997; Sims and Gamon, 2002). Many of these spectral indices have been well recognized as optical traits or surrogates of biological traits (Ollinger, 2011; Ustin et al., 2009). High spectral resolution sensors further enhance the estimation of plant biological traits by providing high fidelity data (Ustin et al., 2009), allowing for capturing subtle changes in spectral signatures as a response to changes in plant physiology and phenology (Gamon et al., 1992; Merzlyak et al., 1999; Peñuelas et al., 1995). For instance, hyperspectral reflectance spectra have been successfully used to estimate leaf pigments based on their unique absorption features in the visible region (Peñuelas et al., 1995; Sims and Gamon, 2002; Ustin et al., 2009) and to estimate leaf water content based on the water absorption features in the near infrared (NIR) and short-wave infrared (SWIR) spectral regions (Eitel et al., 2006; Gao, 1996; Gutierrez et al., 2010; Serrano et al., 2000). Facing the challenge of climate change mitigation, rapid detection of plant water stress using reflectance spectra is becoming increasingly critical (Maimaitiyiming et al., 2017; Zhang et al., 2017).

Leaf chlorophyll fluorescence (ChlF) is part of the light energy dissipated by non-photochemical processes, mainly in the form of red and far-red radiation (Buschmann, 2007; Lichtenthaler et al., 1998). Biotic or abiotic stresses often affect the photosynthetic performance of a leaf, and thus alter the intensity of ChlF emitted from the leaf (Buschmann, 2007; Lichtenthaler et al., 1998). Similar to leaf reflectance spectra, optical traits extracted from a leaf ChlF emission spectrum are widely used to estimate plant health status (Delalieux et al., 2009). Furthermore, combined use of reflectance- and fluorescence-based optical traits allows for a simultaneous quantification of multiple changes in leaves and plants (Delalieux et al., 2009; Yu et al.,



**Fig. 1.** Boxplots showing the differences between the *boxed*, *linear* and *unlimited* planting conditions in: A) specific leaf area (SLA), B) leaf mass per area (LMA), C) leaf water content (LWC) and D) leaf water per area (LWA). Significance levels: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , n.s., not significant.

2014). However, understanding of the feasibility of using leaf ChlF- and reflectance-based optical traits for measuring tree health status under varying planting conditions and for charactering plant strategies in urban environments is still limited.

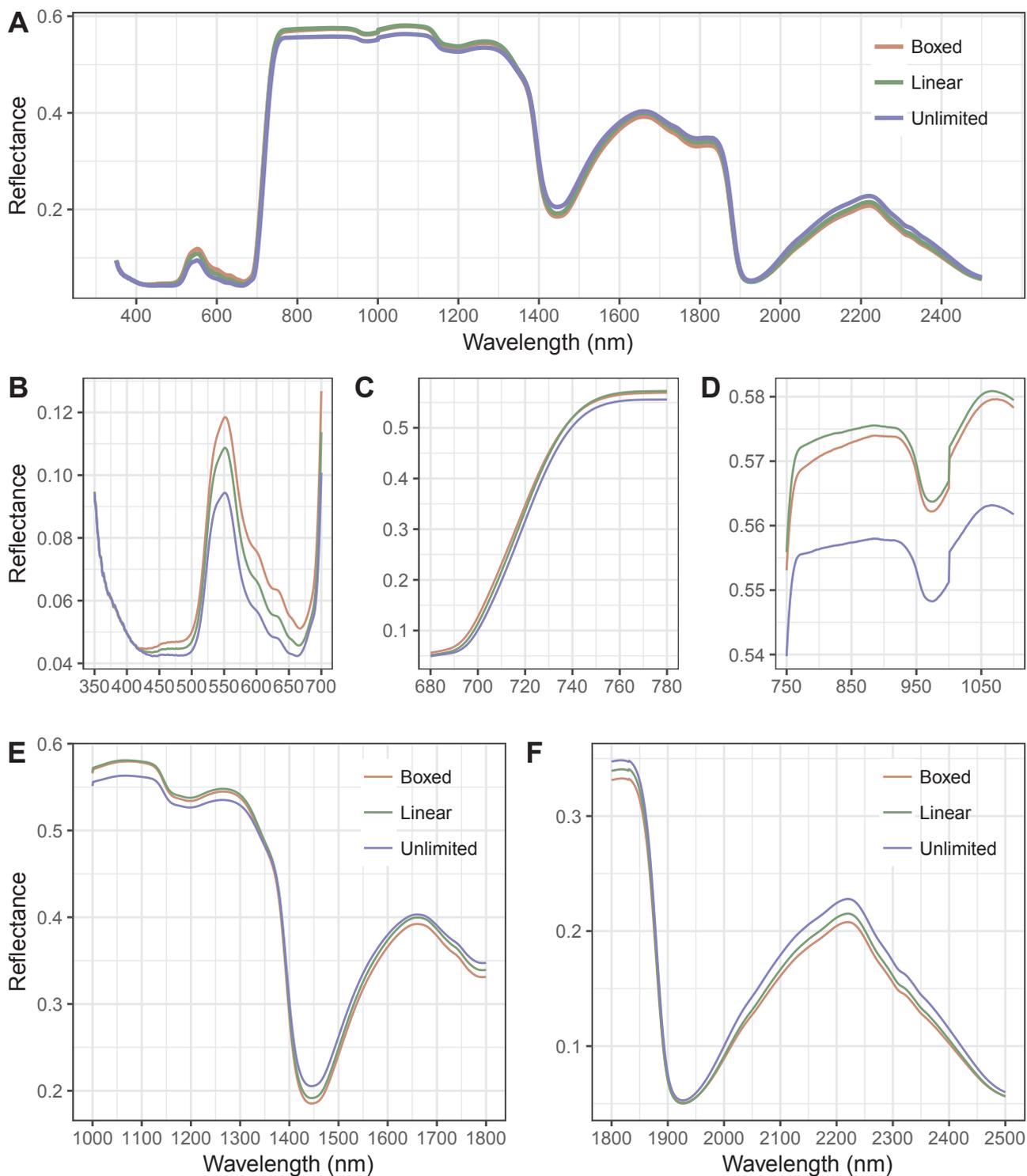
Here, we measured a set of leaf functional traits, leaf ChlF- and reflectance-based optical traits in 187 *Tilia tomentosa* trees growing in urban areas, across three European countries. Planting conditions were visually classified based on different types of sealed soil surface, reflecting a gradient in soil volume and water availability. Our objectives were (i) to examine the effect of planting conditions on leaf functional traits, optical traits and tree phenology; and (ii) to estimate functional traits and tree health status using leaf optical traits.

## 2. Materials and methods

### 2.1. Experimental design and materials

*Tilia* species have been often used as bio-indicator of urban environments (Khavanin Zadeh et al., 2013; Petrova et al., 2014). Here, we used *Tilia tomentosa* as a model species, which is one of the naturally growing *Tilia* species in Europe, commonly planted in urban environments (Radoglou et al., 2009). *T. tomentosa* is a fast-growing, vigorous and heat-and drought-tolerant tree species (Radoglou et al., 2009; Sjöman and Oprea, 2010), and it is moderately shade tolerant in terms of light requirements (Radoglou et al., 2009). *T. tomentosa* flowers between mid-July and early August in Europe.

Including multiple independent samples across different environments allows to avoid experimental bias (Low-Décarie et al., 2014). Therefore, we selected *T. tomentosa* trees growing in three European

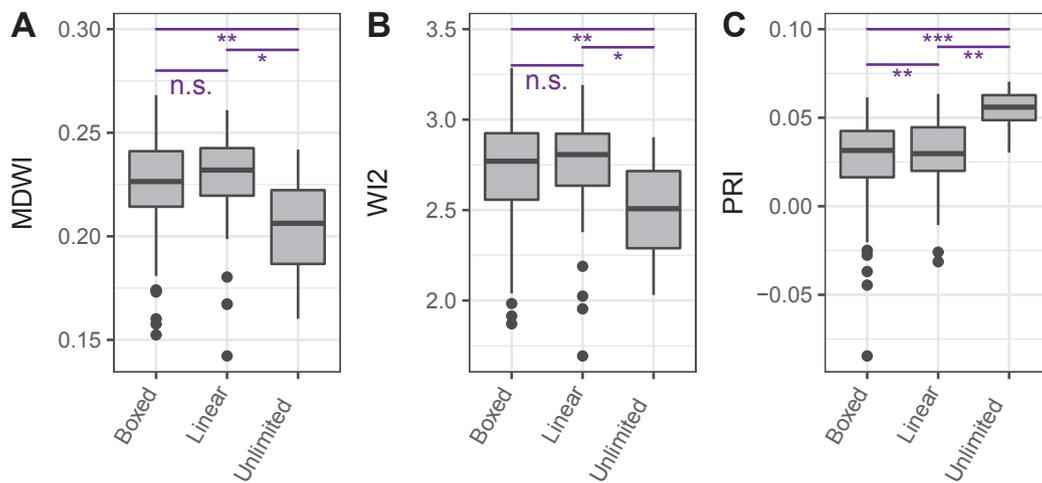


**Fig. 2.** Comparison of leaf reflectance means between the three planting conditions in (A) the full range of 350–2500 nm, (B) visible spectral region, (C) red edge, (D) 750–1100 nm of the near infra-red (NIR), (E) 1000–1800 nm of the short wave infra-red (SWIR1) and (F) 1800–2500 nm of the SWIR2. The mid-row plots share the same legends. Plotted spectra are the means of individual conditions across collections of Leuven and Strasbourg. (For interpretation of colour in this figure legend, the reader is referred to the web version of this article.)

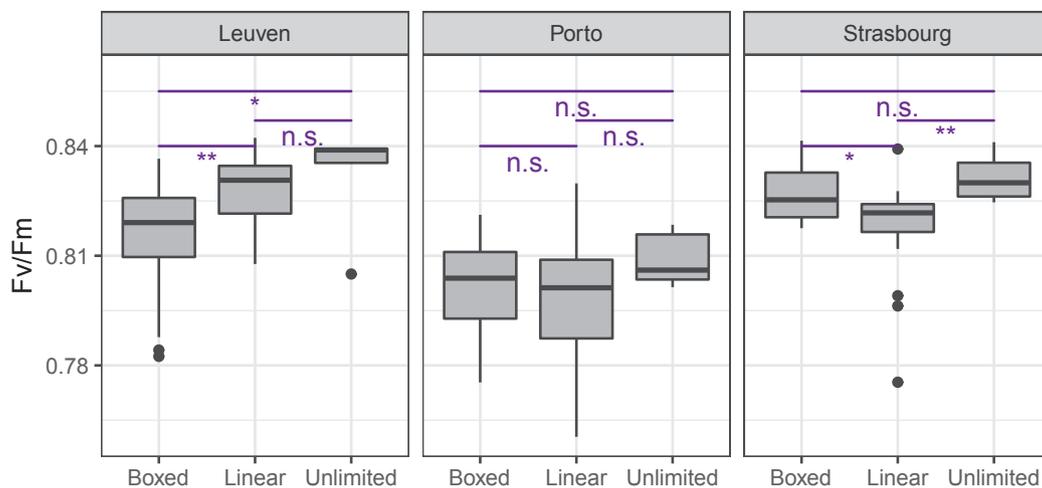
cities (Leuven, Porto and Strasbourg) under three planting conditions, including (i) *boxed*, individual trees planted in a small rectangular area of soil pits surrounded by a very highly sealed surface (concrete, pavement or asphalt); (ii) *linear*, trees planted in a soil strip surrounded by a highly sealed surface; and (iii) *unlimited*, trees planted within a large green area, such as parks. In each city, several sites (streets, parks) were randomly selected in order to include all the three planting conditions

(Table S1). We selected 64, 67 and 56 trees in Leuven, Porto and Strasbourg, respectively, in total 187 trees. Tree diameter at breast height (~1.70 m) was determined by measuring the trunk circumference.

We sampled leaves of the selected trees in each city at a similar development stage (29 August – 28 September 2017) (Dataset 1, Table S2). Additionally, we collected three additional collections in Leuven



**Fig. 3.** Boxplots show the difference between the three planting conditions observed in spectral indices: (A) the maximum difference water index (MDWI), (B) the water index 2 (WI2), and (C) the physiological reflectance index (PRI). Significance levels: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , n.s., not significant.



**Fig. 4.** Boxplots show the Fv/Fm differences between the three planting conditions in each city. Significance levels: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , n.s., not significant.

(13 June – 4 August 2017) in order to study the changes in phenology (Dataset 2, Table S2). For each tree, three randomly chosen branches located respectively within three equally sized sectors (i.e. nadir view) of the crown were detached. Every sampled branch had at least five intact leaves. Sampled branches were stored in a cool box and transported to the lab for leaf reflectance, fluorescence and functional trait measurements. For each tree, 15 selected leaves (3 branches  $\times$  5 leaves per branch) were measured in the following order for: (i) leaf reflectance, (ii) fluorescence, (iii) detaching leaves from the branch and removing petioles for measuring leaf fresh weight (FW) and (vi) leaf area scanned on a flatbed scanner.

Measured leaves were dried at 75 °C for more than 3 days until a constant dry weight (DW). Thereafter, the leaf water content (LWC) was calculated following  $LWC = (FW - DW)/DW$ . Based on the total area of the 15 selected leaves (A), the leaf water per area ( $LWA = (FW - DW)/A$ ), specific leaf area ( $SLA = A/DW$ ) and leaf dry mass per unit area ( $LMA = DW/A$ ) were calculated. Since LMA is the inverse of SLA, correlation analysis will be focused mainly on SLA.

## 2.2. Leaf reflectance measurements

Leaf reflectance was measured using an ASD FieldSpec 3 spectroradiometer (ASD Inc., Longmont, CO, USA) connected to a Plant Probe combined with Leaf Clip Assembly (ASD Inc.). The spectrometer

operates in the spectral range of 350–2500 nm. The Leaf Clip Assembly measures a spot size of  $\sim 10$  mm, within a round clipping area with a diameter of  $\sim 2.5$  cm. On each leaf, three spots on the adaxial side were measured, collecting in total 45 spectra for the 15 selected leaves of each tree. The 45 reflectance spectra were averaged per individual trees for further analyses.

In order to link optical traits to tree health status, we selected the following spectral indices (SIs) related to (i) plant chlorophyll content – mSR705 and mND705 (Sims and Gamon, 2002), (ii) water status – NDWI, MDWI, WI and WI2 (Eitel et al., 2006; Gao, 1996; Peñuelas et al., 1997; Seelig et al., 2008), (iii) photosynthetic light use efficiency – PRI (Gamon et al., 1992), and (iv) plant senescence – PSRI and SIPI (Merzlyak et al., 1999; Peñuelas et al., 1995). Details of calculation formulas of the selected SIs are listed in Table S3.

## 2.3. Leaf chlorophyll fluorescence measurements

We measured leaf ChlF using a Handy PEA fluorometer (Hansatech Instruments Ltd., Pentney, UK) together with specially designed leaf clips with shutter plates that allow to measure a spot size of  $4 \times 4$  mm<sup>2</sup>. On each selected leaf, a random spot was measured after a dark adaptation for  $\sim 25$  min, generating 15 data records for each tree. We used the maximum efficiency of PSII (Fv/Fm) and performance index (PI) and averaged the 15 records per individual trees for further

**Table 2**

Mixed models for testing the effect of planting condition and time interaction on repeated measures of leaf optical traits measured multiple times in Leuven (Dataset 2, see details in Table S2). Bold font highlights the optical traits that capture the phenology difference between planting conditions ( $p < 0.05$ ).

Variable	Factor	F-value	P-value
<b>mSR705</b>	Condition	4.159	0.018
	Time	14.103	< 0.001
	Condition × Time	4.217	<b>0.017</b>
<b>mND705</b>	Condition	5.93	0.004
	Time	14.953	< 0.001
	Condition × Time	5.991	<b>0.003</b>
NDWI	Condition	0.016	0.984
	Time	2.528	0.115
	Condition × Time	0.017	0.984
WI	Condition	0.595	0.553
	Time	1.359	0.246
	Condition × Time	0.596	0.553
MDWI	Condition	0.748	0.476
	Time	0.726	0.396
	Condition × Time	0.758	0.471
WI2	Condition	1.183	0.31
	Time	0.481	0.49
	Condition × Time	1.183	0.31
<b>PRI</b>	Condition	7.179	0.001
	Time	30.379	< 0.001
	Condition × Time	7.255	<b>0.001</b>
<b>PSRI</b>	Condition	3.189	0.044
	Time	3.559	0.061
	Condition × Time	3.191	<b>0.044</b>
<b>SIPI</b>	Condition	3.757	0.026
	Time	5.871	0.017
	Condition × Time	3.769	<b>0.026</b>
Fv/Fm	Condition	0.474	0.624
	Time	2.232	0.138
	Condition × Time	0.482	0.619
PI	Condition	0.933	0.397
	Time	0.339	0.562
	Condition × Time	0.953	0.389

analyses. Fv/Fm is sensitive to a wide range of plant stresses (Chen et al., 2015). PI can be expressed in fluorescence terms, i.e.  $PI = (V_j / (dV/dt)) * F_v/F_m * (F_v/F_o) * (F_m - F_j) / (F_j - F_o)$ , where for a detailed explanation readers are encouraged to refer to Strasser et al. (2000). PI is related to the forces of redox reactions in biochemical systems, and it is an indicator of plant/leaf sample vitality (Strasser and Srivastava, 1995).

#### 2.4. Statistical analysis

We divided the entire dataset into two subsets: Datasets 1 and 2 (Table S2). Dataset 1 was used to test whether planting conditions affect the leaf growth and health of trees. We used linear mixed models to test the effects of the tree planting conditions (fixed factor) on the analyzed leaf functional and optical traits using the variables *city* and *site* as random effect factors. In addition, Dataset 2, including four collections in Leuven only, was used to test whether planting conditions have an impact on tree phenology, i.e., testing the effect of planting-condition-by-time interaction on optical traits by applying mixed models for repeated measures.

Pooled data (i.e. datasets 1 & 2, Table S2) were used to determine the potential of optical traits as predictors of leaf functional traits. We used principal components analysis (PCA) to examine the relative contribution of individual variables to the overall variation in traits. For a comparison of variable associations, we also conducted the Pearson's correlation analysis for the relationships between individual variables

as well as for correlations between the optical traits and functional traits. Statistical analyses were performed in the R programming environment (R Core Team, 2016). We used the package 'lme4' (Bates et al., 2015) for running the mixed models, and the package 'lsmeans' (Lenth, 2016) for post-hoc analysis comparing planting conditions pairwise based on Tukey's HSD test.

### 3. Results

#### 3.1. Planting condition effect on leaf functional traits

Planting conditions had significant effects on the measured functional traits ( $p < 0.05$ , Table 1). According to post-hoc tests for pairwise comparisons, the *boxed* and *linear* trees were significantly different from the *unlimited* trees, whereas we did not observe significant differences between the *boxed* and *linear* trees, except for LWC (Table 1). SLA in the *boxed* and *linear* trees was significantly lower than in the *unlimited* trees (Fig. 1A, Table 1), whereas LMA was significantly higher than in the *unlimited* trees (Fig. 1B, Table 1). LWC in the *boxed* and *linear* trees was significantly lower than in the *unlimited* trees (Fig. 1C, Table 1), whereas LWA was significantly higher than in the *unlimited* trees (Fig. 1D, Table 1).

#### 3.2. Planting condition effect on leaf optical traits

Leaf reflectance in the visible region in the *boxed* and *linear* trees was higher than in the *unlimited* trees, and it was highest in the *boxed* trees, followed by the *linear* ones (Fig. 2A-B). The Leaf reflectance in the *unlimited* trees were lower than the *boxed* and *linear* trees in the NIR (750–1300 nm) (Fig. 2C-E). In contrast, the reflectance in the SWIR regions (1300–2500 nm) were higher in the *unlimited* trees, and it was lowest in the *boxed* trees, followed by the *linear* trees (Fig. 2E-F).

Planting conditions had a significant effect on mSR705, ND705, MDWI, WI2 and PRI ( $p < 0.01$ , Table 1), among other SIs. MDWI, WI2 and PRI in the *boxed* and *linear* trees were significantly different from the *unlimited* trees ( $p < 0.05$ ), whereas the differences between the *boxed* and *linear* trees were relatively small and PRI was the only spectral index that showed significant difference (Fig. 3, Table 1). Moreover, PRI was the only spectral index that indicated the significant difference between all the planting conditions.

For ChlF traits, we observed a significant planting condition effect on PI ( $p < 0.01$ , Table 1) rather than on Fv/Fm ( $p = 0.07$ ). For pairwise comparisons, PI values of the *boxed* and *linear* trees were significantly lower than the *unlimited* trees. In contrast, there were no significant Fv/Fm differences between planting conditions. Yet, we observed lower Fv/Fm in the *boxed* and *linear* trees than in the *unlimited* trees in each city individually (Fig. 4, Table 1).

We found a significant planting-condition-by-time interaction effect on mSR705, ND705, PRI, PSRI and SIPI ( $p < 0.05$ , Table 2), compared to other optical traits, and PRI was the best performing one ( $p \leq 0.001$ ). The mSR705, ND705, PRI, PSRI and SIPI showed rapidly changing trends in the *boxed* trees compared to the *unlimited* trees (Fig. 5). Specifically, the mSR705, ND705 and PRI showed a faster decline in the *boxed* trees than in *unlimited* trees, whereas PSRI and SIPI showed a steeper increase in the *boxed* trees than in the *unlimited* trees.

#### 3.3. Relationships between functional traits and optical traits

PCA showed that the trunk diameter did not account for much of the trait variations (Fig. 6A). Fv/Fm, PI and NDWI accounted for most of the variations in the first dimension, followed by mSR705, mND705, MDWI, WI2 and PRI. SIPI was independent of SLA and LMA compared to other SIs. LWC was weakly associated with water SIs, instead it was closely related to PRI. Water SIs were more dependent of LWA than of LWC (Fig. 6A), which is also indicated by their correlation coefficients (Fig. 6B).

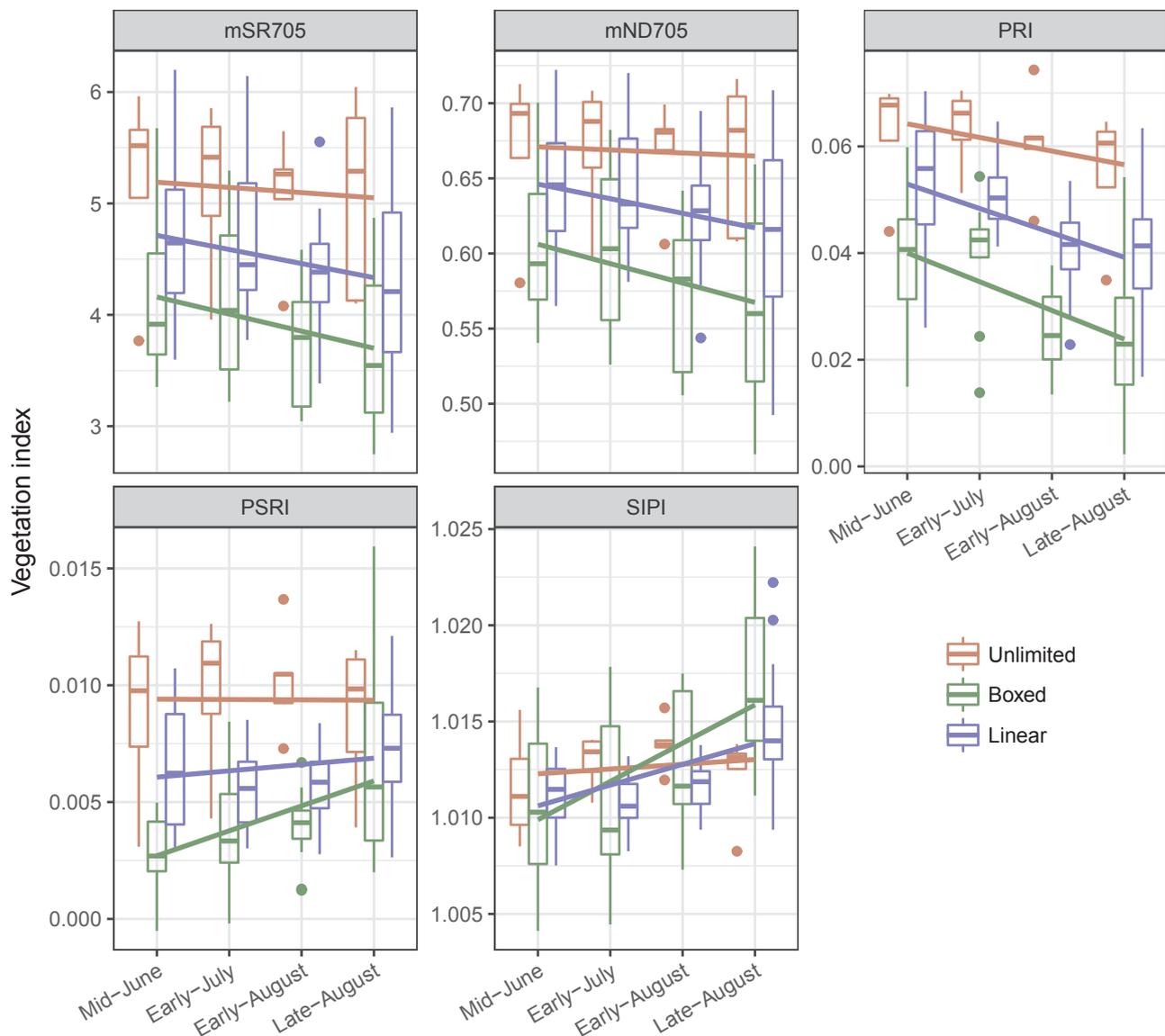


Fig. 5. Spectral indices plotted as a function of collection time per planting condition, showing the different changes in phenology between planting conditions. (For interpretation of colour in this figure legend, the reader is referred to the web version of this article.)

SLA and LMA correlated strongly with MDWI and WI2, followed by PRI and PSRI, WI and NDWI (Fig. 6B). LWC and LWA showed contrasting relationships with SIs. LWC was highly correlated with PRI, followed by NDWI, mSR705 mND705 and PSRI. In contrast, LWA correlated strongly with water SIs, i.e., MDWI, WI2, NDWI and WI. SIPI, however, was not correlated with SLA, LMA, LWC and LWA (Fig. 6B).

Correlations between the leaf functional traits and their corresponding highest ranked four SIs are illustrated in Fig. 7. MDWI and WI2 yielded the best correlations with SLA ( $r = -0.79$ ,  $p < 0.001$ , Fig. 7A). PRI yielded the best correlation with LWC ( $r = 0.66$ ,  $p < 0.001$ , Fig. 7B), followed by PSRI ( $r = 0.34$ ,  $p < 0.001$ ). MDWI, NDWI, and WI2 yielded a same magnitude of correlations ( $p < 0.001$ ), whereas WI was not correlated with LWC (Fig. S1). In contrast, MDWI and WI2 yielded the best correlations with LWA ( $r = 0.88$ ,  $p < 0.001$ , Fig. 7C), followed by WI ( $r = -0.71$ ,  $p < 0.001$ ) and NDWI ( $r = 0.64$ ,  $p < 0.001$ ).

### 3.4. Development patterns of traits

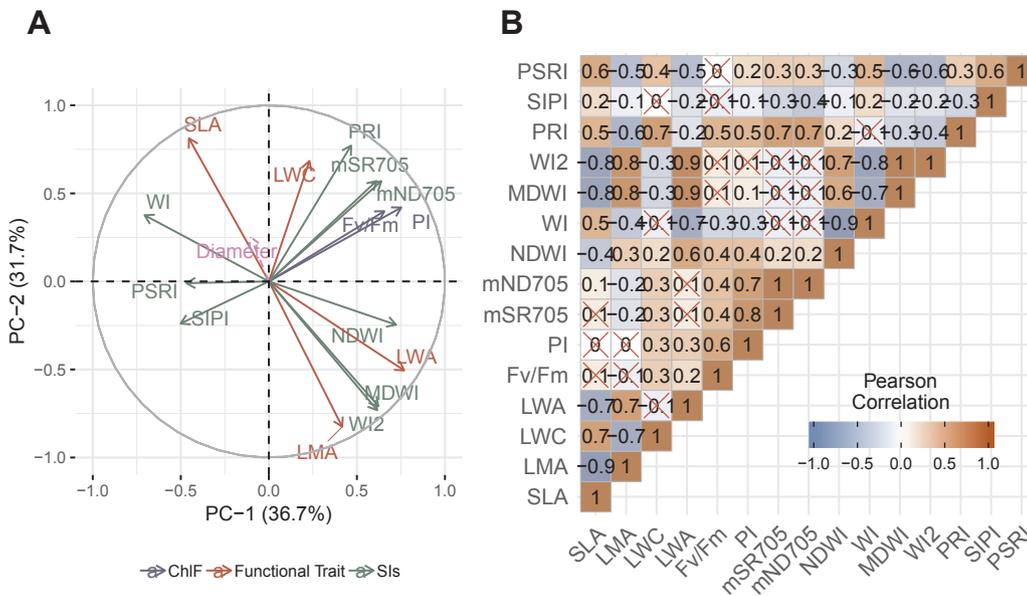
By normalizing the leaf optical traits and functional traits to the same range (0 ~ 1), we observed that trees growing in different

planting conditions displayed distinct patterns of trait variations (Fig. 8). Compared to the *unlimited* trees, the *boxed* and *linear* trees produced low scores of SLA and LWC and high scores of LMA and LWA. The *unlimited* trees showed high scores of mND705, mSR705, PRI and WI, whereas the *boxed* and *linear* trees showed high scores of MDWI, NDWI, SIPI and WI2.

## 4. Discussion

### 4.1. Tree health status reflected in functional trait variations

Decreased SLA and LWC in the *boxed* trees suggest that soil sealing affects leaf growth, which can be attributed to small soil volumes and low water availability in sealed soils. This is in agreement with other studies reporting that drought causes decreased SLA (Farooq et al., 2009; Fernández et al., 2002; Xu et al., 2009; Zhang et al., 2015). Varying effects of water stress on SLA, however, might exist in different climate zones or due to intraspecific variations – for instance, forbs may not show decreased SLA under drought in temperate systems (Wellstein et al., 2017). Decreased SLA in *boxed* trees implies a reduction of leaf area, which has been found in many species as an adaptation to drought



**Fig. 6.** (A) Relationships between three trait groups (ChlF: chlorophyll fluorescence, SIs: spectral indices), trait variables as well as their contributions to explaining the variations: axis x and y represented the first and second components. (B) Relationships between individual traits are determined using Pearson's correlations. The red cross indicates statistically non-significant correlations, or otherwise is statistically significant ( $P < 0.05$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Xu et al., 2009; Zhang et al., 2015). One of the main reasons of SLA reduction during drought is that leaf expansion and translocation of assimilates from leaf to stem might be inhibited, resulting in thicker leaves and lower SLA (Zhang et al., 2015). Drought induced SLA reduction may further cause reduced whole-plant growth and biomass production (Fernández et al., 2002). Similarly, in urban environments, trees planted in pits often have small canopies compared to trees planted in soil strips (Sanders and Grabosky, 2014).

It has been found that LMA reduces along with water availability across a range of control environments (Poorter et al., 2009), and that across a range of natural environments with lower water availability plants are generally characterized by a high LMA (de la Riva et al., 2016). However, less usable soil and water stress may not be the only factors causing the variations in LMA. LMA also varies with nutrient, light availability and other environmental factors (Witkowski and Lamont, 1991; Xu et al., 2009). It is very likely that the boxed trees have low nutrient availability given that highly sealed soils often constrain nutrient supplying and holding capacity (Smiley et al., 2006). Urban soil sealing reduces not only the soil carbon and nitrogen content but also the microbial biomass and its activity (Piotrowska-Długosz and Charzyński, 2015; Zhao et al., 2012), which in turn affects root and tree growth and functional traits. Under varying light availability, plants may optimize canopy photosynthetic rate by investing differently in LMA. For instance, with a steeper leaf inclination, the maximum of canopy photosynthesis rate is often reached at a lower LMA (Gutschick and Wiegell, 1988).

#### 4.2. Water stress indicated by optical traits

Decreased LWC and increased LWA in the boxed and linear trees confirm that the highly sealed soils have low water availability, causing water stress. NDWI and WI are closely related to LWC of plants growing under severe drought stresses (Eitel et al., 2006; Gutierrez et al., 2010; Peñuelas et al., 1997). NDWI and WI were highly correlated with LWA, despite the relatively weak correlations with LWC. Similarly, a previous study reported that NDWI and WI were not correlated with LWC under low-to-moderate water stress (Eitel et al., 2006). Therefore, our results imply that the degree of water stress in the boxed and linear trees is not likely to be severe or persistent, and that NDWI and WI may not be the optimal surrogates of LWC when water deficit is not very severe. Surprisingly, PRI was highly correlated with LWC, suggesting that short-term plant physiological changes in response to water stress can be

detected using PRI (Gamon et al., 1992; Maimaitiyiming et al., 2017).

Relatively weak relations between the water SIs and LWC can be attributed the confounding effect of leaf thickness on leaf reflectance (Eitel et al., 2006; Seelig et al., 2008). A recent study showed that the NIR reflectance of a leaf stack is about 25% higher than that of a single leaf (Neuwirthová et al., 2017), suggesting that thicker leaves produce high NIR reflectance. The high NIR reflectance observed in the boxed and linear trees suggests an increased leaf thickness (cf. Fig. 2). SWIR reflectance might be more sensitive to leaf water status than NIR reflectance (Eitel et al., 2006; Seelig et al., 2008). By employing SWIR bands, MDWI and WI2 improved the correlation with LWA compared to NDWI and WI, suggesting that SWIR reflectance is highly sensitive to leaf water content while less sensitive to leaf thickness at leaf level (Eitel et al., 2006). From the perspective of remote sensing, LWA measured with reflectance data can be very robust compared to LWC, since LWA partially accounts for the leaf water thickness. Yet, leaves may develop a certain leaf thickness even under no water deficit (Seelig et al., 2008). Considering the scale effect, NIR may be suitable for water estimation from a distance above the canopy (Gutierrez et al., 2010; Serrano et al., 2000). Overall, measured optical traits indicate that sealed soils pose water stress on trees, particularly in dry seasons (Sela et al., 2015). Further investigations are needed to study how leaf thickness and other factors affect water estimation using hyperspectral reflectance data.

#### 4.3. Senescence and phenology change indicated by optical traits

Significant planting-condition-by-time effects on SIs is highlighted by the rapidly changing trends of SIs in the boxed trees compared to unlimited trees, suggesting that soil sealing limits soil water availability and in turn affects tree phenology (Link et al., 1990; Xie et al., 2015). SIPI is an indicator of changes in carotenoids relative to chlorophyll, and it is related to senescence (Peñuelas et al., 1995). Steeply increased SIPI in the boxed trees suggests that soil sealing accelerated leaf senescence. PSRI is also related to the relative changes in carotenoids and chlorophyll (Merzlyak et al., 1999), but its changes appeared to be more pronounced at individual time points rather than in the temporal trends observed here. Compared to PSRI, SIPI was independent of the variations in functional traits (cf. Fig. 6). Therefore, considering the highly varying leaf structures across multiple species, SIPI can be used as a robust indicator for early identification of senescence compared to other SIs.

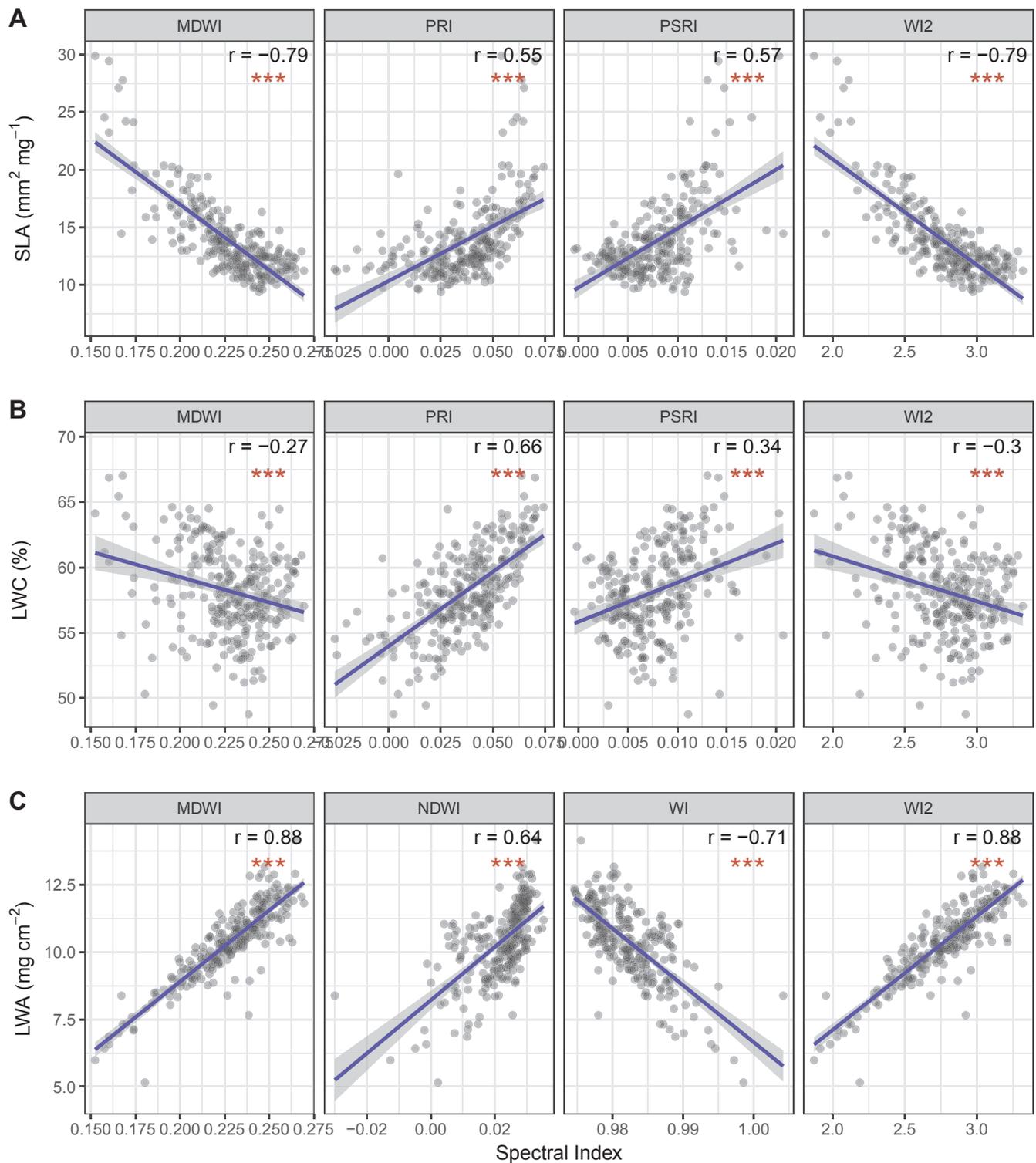
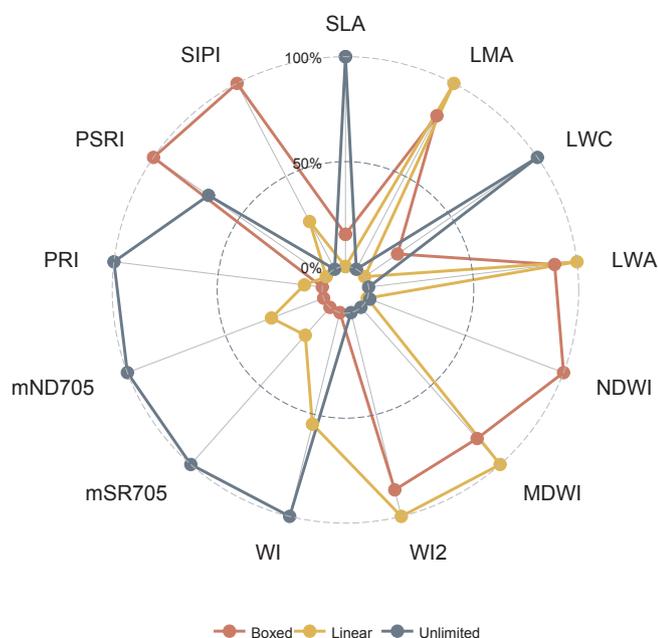


Fig. 7. Correlations between the selected spectral indices and (A) specific leaf area (SLA), (B) leaf water content (LWC) and (C) leaf water per area (LWA). Significance levels: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ . Scatter plots are illustrated with transparency to display the clustering pattern of data points.

Rapid decline in mSR705 and mND705 for the boxed trees are associated with chlorophyll degradation, whereas decline in PRI may be associated with photosynthetic activity and physiological responses to water stress. The boxed trees grow under low water availability, which can be the direct cause of early onset of leaf senescence since drought accelerates senescence (Chen et al., 2015; Estiarte and Peñuelas, 2015; Xie et al., 2015). At global scale, global warming may accelerate the growth of urban trees or delay leaf senescence (Estiarte and Peñuelas,

2015; Pretzsch et al., 2017). From a different perspective and scale, our results showed that planting conditions have a large impact on functional traits and phenology in a relatively consistent manner across three European cities. Our results raise the need of studying the effect of climate change on urban tree growth by taking into account planting conditions.



**Fig. 8.** Radar plots show the normalized performance metrics of the *boxed*, *linear* and *unlimited* trees in the development of the given leaf functional and optical traits. (For interpretation of colour in this figure legend, the reader is referred to the web version of this article.)

#### 4.4. Characterization of plant strategies using multiple optical traits

Leaf area-based traits such as SLA and LWA can vary as a result of changes in leaf thickness, particularly across species, in different climate zones, suggesting that one cannot predict plant strategies perfectly using a single trait (Wellstein et al., 2017; Wilson et al., 1999). Our results show that the selected SIs have reliable performance in estimating different functional traits and differentiating planting conditions. Therefore, the selected SIs provide a means to rapidly estimate leaf morpho-physiological and biochemical variations that are associated with the development of certain strategies mitigating negative effects due to soil sealing in urban environments.

ChlF metrics differed slightly in distinguishing sealed planting conditions in three cities, and we did not observe significant effects of planting conditions on Fv/Fm across cities. This can be attributed to, on one hand, that Fv/Fm is sensitive to severe stress which was not likely the case in this study; and on the other hand, that the difficulty of ChlF measurements lies in the fact that it was inapplicable to determine the optimal dark adaptation time for individual planting conditions, sites or cities. Typically, ChlF measurements need a dark adaptation preceding the measurement, which increases the time cost when measuring a large amount of samples compared to leaf reflectance measurements. In contrast, reflectance-based approach simplifies the measurement procedure while maintains the power of capturing subtle variations over a range of narrow bands, highlighting that leaf optical traits extracted from hyperspectral reflectance are indicative of moderate stresses and unfavorable growing conditions for urban trees.

By combining the hyperspectral optical traits and functional traits, plant developmental strategies can be characterized for individual planting conditions. Distinct development patterns produced by the three planting conditions highlight the developmental strengths and weaknesses in functional traits and hyperspectral optical traits of trees (Fig. 8). For instance, possessing low scores of SLA and LWC and high scores of LMA and LWA, the *boxed* and *linear* trees show the characteristics of water stress. Clearly, the development strategy is embodied in the dissimilarities in hyperspectral optical traits, which thus enables to characterize the structure and function of urban vegetation

as a whole, in a spatially explicit manner (Alonzo et al., 2016). For instance, SIPI (i.e. carotenoid-chlorophyll ratio) has been used as an indicator of urban green roof vegetation health status (Piro et al., 2017). Here, the high scores of SIPI highlight the association with a high degree of leaf chlorosis and senescence, and the concurrently observed low scores of PRI indicate a relatively low investment in photosynthetic activity (cf. Fig. 8). Collectively, optical traits are able to detect the negative effects of planting conditions and to estimate leaf functional traits, and thus have great promise for characterizing plant strategies.

Among other spectral indices tested in this study, PRI was the best performing spectral index in differentiating planting conditions as well as for capturing the phenology changes. In addition to PRI, the use of multiple optical traits is recommended for assessing urban vegetation health status. For detecting soil sealing stress particularly, mSR705, mND705 and the water spectral indices using SWIR bands, MDWI and WI2, are recommended; and SIPI is recommended for detecting leaf phenology change.

Further research needs to determine the best strategy for the assessment of urban tree health using optical and multimodal sensing techniques (Alonzo et al., 2016; Degerickx et al., 2018). This will enable us to develop a standardized quantitative approach for monitoring and assessing tree health status across multiple environments, as well as for up-scaling using air- and space-borne remote sensing data (Degerickx et al., 2018; van der Linden et al., 2018).

## 5. Conclusions

This study investigated how the varying planting conditions in urban areas affect tree health status by measuring a set of foliar functional traits and optical traits of *Tilia tomentosa* trees, across multiple environments in three European countries. We examined whether the variations in functional traits are predictable by non-destructively measured foliar optical traits, particularly those spectral indices related to leaf chlorophyll, water, photosynthetic efficiency and senescence. Results showed that highly sealed planting conditions have negative impacts on urban tree health status, yielding decreased specific leaf area and leaf water content and accelerated leaf senescence. Non-destructively measured foliar optical traits can predict these negative effects, such that PRI and MDWI explain 60%–80% of the variations in specific leaf area and leaf water status; and that SIPI is associated with advanced leaf senescence while being irrespective of other functional traits, demonstrating the power of combining multiple optical traits for monitoring urban tree health status. Our findings imply strong associations between planting conditions and health status of urban trees, and provide new insights into urban green management for the provisioning of ecosystem services.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ecolind.2018.08.047>.

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