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Effects of artificial light at night on the leaf functional traits of freshwater plants

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Abstract

1. The increasing use of artificial light at night has led to ecosystem exposure to light pollution worldwide. Aquatic ecosystems are particularly exposed, since lit road networks, urban development and industrial infrastructure are frequently located along river, lake, and sea shores. Although the negative effects of nighttime lighting on the physiology, behaviour, and life-history traits of animals have been largely documented, there is a large knowledge gap about the responses of plants, especially regarding leaf functioning and resource-management strategies. Some authors have proposed contrasting hypotheses of mechanistic responses to dim light at night in plants, but empirical results are still lacking.
2. Based on field measurements of nocturnal irradiance in freshwater ecosystems located in peri-urban areas, we performed a mesocosm experiment using three species of submerged aquatic plants. After 5 months of exposure to realistic dim light at night, four functional traits related to the resource management at the leaf level were measured.
3. Artificial light at night had significant effects on the leaf physiology or chemistry, affecting their resource acquisition rate, but with different response intensities depending on the species. No effect was found on morphological or biomechanical traits for any of the studied species.
4. These results support the hypothesis that plants could interpret dim light at night as a shaded environment and respond accordingly.
5. We demonstrated that the effects of light at night on plants may have been underestimated in previous work. By modifying biotic interactions (e.g., competition and herbivory), these responses can have profound effects on community structure and ecosystem functioning.

KEYWORDS
artificial light at night, freshwater ecosystems, leaf economics, plant trait response, submerged aquatic vegetation

1 | INTRODUCTION

Artificial light at night (ALAN hereafter) is now recognised as an anthropogenic pollutant of concern, jeopardising biodiversity at a global scale (Gaston et al., 2015; Guetté et al., 2018; Hölker et al., 2010; Sanders et al., 2020; Secondi et al., 2020). Falchi et al. (2016) showed that 23% of the world's land surfaces between 75°N and 60°S, 88% of Europe, and almost half of the U.S.A. experience light-polluted nights. In these areas, ALAN increases the brightness of the night and alters the illumination regime beyond the natural range. Moreover, areas impacted by ALAN are likely to continue to increase in the near future, further eroding Earth's remaining land area that experiences natural day–night light cycles (Kyba et al., 2017). The negative effects of ALAN on animal physiology, behaviour, and life-history traits have been largely documented in the last 2 decades (Desouhant et al., 2019; Gaston et al., 2017; Longcore & Rich, 2004; Owens & Lewis, 2018; Rich & Longcore, 2006; for reviews), but surprisingly, many fewer studies have focused on vascular plants (but see Bennie et al., 2016; Briggs, 2006; ffrench-Constant et al., 2016; Massetti, 2018).

In plants, ALAN can alter the perception of seasonal changes in day length. Photoperiod is a critical environmental cue that enables many plant species to control their phenology (Briggs, 2006; and references therein). A few studies have reported changes in the onset of flowering, leaf fall, bud dormancy, and bud burst for trees growing in areas exposed to urban light at night (Bennie et al., 2016; Cathey & Campbell, 1975a; ffrench-Constant et al., 2016; Massetti, 2018). Škvareninová et al. (2017) even reported variations in the onset of leaf fall between branches of the same tree depending on their exposure to streetlights for *Rhus typhina* and *Acer pseudoplatanus*. However, these responses differ strongly between species depending on their sensitivity to photoperiod variations (see Cathey & Campbell, 1975b; for classification of tree species according to their sensitivity to ALAN). These results were mostly found under relatively high intensities of ALAN representative of direct exposure to streetlights.

Another potential effect of ALAN on plants that has been poorly investigated to our knowledge concerns leaf physiological activity at night and its consequences on resource allocation and plant growth. Although the intensity of ALAN is several orders of magnitude lower than that of daylight, it has been suggested that it could support a marginally beneficial amount of additional photosynthesis by the plant (Briggs, 2006). Moreover, Briggs (2006) hypothesised that low intensity of night-time light and the different spectral composition compared to that of direct sunlight could be interpreted as a shaded environment by plants. This would induce faster growth, a higher leaf extension rate and a higher photosynthesis rate (Briggs, 2006). Conversely, it has also been proposed that ALAN could alter the circadian clocks of plants, especially those involved in the repair and recovery of physiological processes that generally occur in the dark period (Gaston et al., 2013; Velez-Ramirez et al., 2011). These effects could result in damage to the photosynthetic machinery, leading, for example, to reduced photosynthetic capacities. Furthermore, these contrasting hypotheses are associated with discrepancies in experimental results: a higher photosynthetic capacity was reported after short exposure to ALAN (≤4 months) in marine autotrophic biofilms (Maggi et al., 2020) and *Liriodendron tulipifera* (Kwak et al., 2018), while opposite results were found after a similar exposure period in red corals (Ayalon et al., 2019) and a long exposure (>1 year) in trees (Kwak et al., 2018; Meravi & Prajapati, 2020). However, these results require nuanced interpretation since most of the experiments described above simulated high-intensity night illumination (photosynthetically active radiation [PAR] >1 μmol m^{-2} s^{-1}). Such light levels are rarely found apart from direct exposure to streetlights, and in our opinion, the effects of ALAN must be tested in other ecological contexts. Indeed, light pollution extends over tens of kilometres around urban centres at much lower light intensities (Kyba et al., 2017) due to indirect light reflected from surfaces or scattered in the atmosphere. Thus, most ecosystems in peri-urban to rural areas experience dim nocturnal light, potentially affecting the communities they shelter (Bennie et al., 2016; Gaston et al., 2015). For example, Poulin et al. (2014) measured illumination of 0.08 μmol m^{-2} s^{-1} (*c.* 6.6 lx) on the shore of a lake in Sherbrooke (Quebec, Canada) and demonstrated no effect of this night-time light pollution on cyanobacteria photosynthetic capacities (but see Poulin et al., 2014 for other physiological responses).

Therefore, there is a large gap in knowledge about plant responses to ALAN, especially regarding leaf functioning. It is still unclear whether the intensity of ALAN in peri-urban areas (PAR <0.1 μmol m^{-2} s^{-1}) has an effect on plant leaf traits. Freshwater ecosystems are particularly exposed to ALAN (Bennie et al., 2015; Grubisic, 2018; Grubisic et al., 2018; Secondi et al., 2017) since lit road networks, urban development and industrial infrastructure are frequently located along river, lake, and sea shores (Reid et al., 2019). Moreover, aquatic plant species (especially submerged species) can colonise habitats with low light levels and be confronted with strong variations in light availability due to changes in water levels and turbidity (e.g. shading by phytoplankton and epiphyton, Bornette & Puijalon, 2011). Therefore, one can expect these species to be particularly responsive to low light intensity, such as that from ALAN pollution.

Here, we performed a mesocosm experiment using three freshwater macrophyte species to address this question. To our knowledge, this is the first study testing the effect of realistic intensities of ALAN representative of peri-urban areas (0.05 μmol m^{-2} s^{-1}) on leaf functioning. For comparison purposes, we measured night-time PAR values of 0.065 ± 0.003 μmol m^{-2} s^{-1} on the Rhône River (Passerelle du collège 45.765° N, 4.84° E) and 0.011 ± 0.001 μmol m^{-2} s^{-1} on an urban park pond (Parc de la Tête d'Or, 45.778° N, 4.85° E) in Lyon, France (see Figure S1 for details and other examples). In the present study, we measured traits describing key functions of leaves and covering physiological, morphological, biomechanical, and chemical aspects of plant leaves. We tested whether the leaves produced under dim ALAN differ from controls in their photosynthetic capacities, mass per area, toughness, and carbon to nitrogen ratio (see Table 1 for

TABLE 1 Four leaf traits are used to describe different leaf functions related to morphological, biomechanical, physiological, and chemical properties (see Garnier et al., 2016 for details)

Trait	Leaf function	Abbreviation	Unit
Leaf mass per area	Morphology: light capture per unit biomass	LMA	g/m^2
Specific work to shear	Biomechanics: defence against biotic and abiotic hazards (e.g., herbivory, water flow)	Toughness	kJ/m^2
Maximum photosynthetic rate per leaf area	Physiology: photosynthetic capacity	A_{area}	$\mu mol\ O_2\ m^{-2}\ s^{-1}$
Carbon to nitrogen ratio	Chemistry: carbon-based structural investment compared to nitrogen-based proteins related to metabolism, chemical quality for herbivores	C:*N*	-

functional significance). Fast growth is generally associated with a higher resource acquisition rate but lower investment in structural compounds at the leaf level (Reich, 2014; Wright et al., 2004), especially in herbaceous species. This allocation trade-off results in a higher photosynthetic rate being associated with tender mechanical properties (Onoda et al., 2011) and a lower proportion of carbon-based structural compounds than nitrogen-based proteins involved in metabolism. Therefore, under the hypothesis that ALAN induces faster plant growth, we predict that leaves produced under these conditions have higher photosynthetic capacities, lower mass per area, tender mechanical properties and a relatively low carbon to nitrogen ratio.

2 | METHODS

2.1 | Study design

A mesocosm experiment was conducted on three macrophyte species, namely, *Myriophyllum verticillatum* L. (eudicot of the *Haloragaceae* family), *Potamogeton coloratus* Hornem. (monocot of the *Potamogetonaceae* family), and *Vallisneria spiralis* L. (monocot of the *Hydrocharitaceae* family). Thirty ramets of each species were sampled in the Ain and Rhône Rivers (eastern France) in November 2019. The ramets of each species were collected in different vegetation patches found in the selected sites. They were kept in artificial channels fed with groundwater located next to our lab before the experiment. In January 2020, the ramets were transplanted in 45-L plastic tanks (40 × 34 × 36 cm) containing 6 cm of growth substrate (75% 0.4 mm quarry sand from the Saône River and 25% commercial substrate for aquatic plants with 3% organic matter) and filled with tap water. The substrate provided nutrients for plant growth and no fertiliser was added during the experiment. Although nutrients were not monitored, we did not notice signs of nutrient limitation on the plants during the experiment. We decided to use tap water, which composition is stable through time, to avoid heterogeneity between mesocosms. The experiment was conducted in a climate-controlled room with the room temperature set at 21°C. Air pumps were used to avoid static conditions in the mesocosm and limit development of biofilm. Algae developing at the plant surface were manually removed weekly to avoid competition for light and nutrients.

Five ramets per species were transplanted into each tank. Three tanks per species were used as controls, and three tanks were exposed to ALAN according to the following experimental design. The 18 experimental tanks were placed on six shelves (one tank per species per shelf). On each shelf, the positions of the tank assigned to each species were randomised. The shelves were individually covered with black rubber canvas, preventing light from entering. Daylight (from 08.00 to 18.00) conditions were created by 11 LED ribbons (Vegeled©, Pandora) placed above the tanks, providing a PAR of 450 $\mu mol\ m^{-2}\ s^{-1}$ (*c*. 12,000 lx, Figure S2 for light spectrum) at the water surface. At night (from 18.00 to 08.00), the three shelves assigned to the ALAN treatment were illuminated at a low irradiance of 0.05 $\mu mol\ m^{-2}\ s^{-1}$ (*c*. 3 lx, Figure S2) using two additional LED ribbons (white hot waterproof Light Plus, SC-TWF-WW2) while controls were not illuminated (irradiance at night <0.002 $\mu mol\ m^{-2}\ s^{-1}$; *c*. 0.01 lx).

2.2 | Trait measurements

After a growth period of 5 months (24 January–20 July 2020), the plants were harvested progressively to perform trait measurements. By that time, the three species had produced many new ramets and colonised the entire tank surface. All traits were measured on new leaves produced during the experiment.

The photosynthetic capacity was measured on four ramets collected randomly from each tank. For each ramet, one to three young but fully developed leaves were selected according to leaf size and were placed immediately after collection in a closed chamber device (adapted from Pedersen et al., 2013) to measure their maximum photosynthetic rate per leaf area ($\mu mol\ O_2\ m^{-2}\ s^{-1}$, A_{area} hereafter). Underwater photosynthesis was monitored by optodes based on the production of O_2 under conditions not limited by light (PAR > 1,200 $\mu mol\ m^{-2}\ s^{-1}$) or CO_2 availability. A detailed description of the device and the protocol can be found in Appendix S1. After the gas exchange measurements, image analysis of leaf scans was used to measure leaf area (WinFolia 2006a, Regent Instruments Inc., Quebec, Canada); then, the leaves were weighed after oven drying at 65°C for 48 hr to measure their dry mass. The leaf mass per area (g/m^2, LMA hereafter) was calculated as the ratio between the leaf dry mass and the leaf area (Pérez-Harguindeguy et al., 2013).

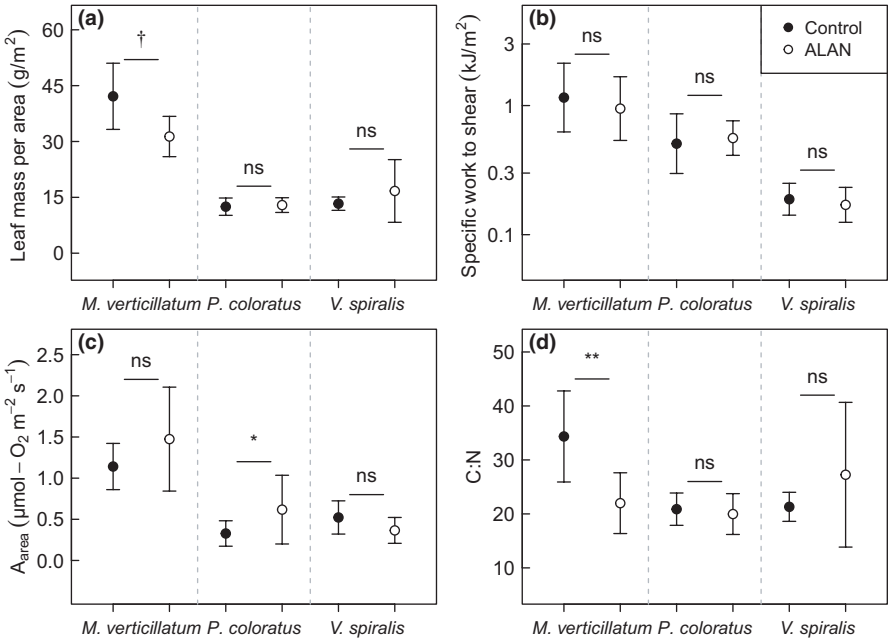

FIGURE 1 1 Leaf traits of the aquatic plant species (*Myriophyllum verticillatum*, *Potamogeton coloratus*, and *Vallisneria spiralis*) grown either under control conditions (closed symbols) or under the artificial light at night (ALAN) treatment (open symbols). (a) Leaf mass per area, (b) specific work to shear or leaf toughness, shown on a $\log_{10}$ axis, (c) maximum photosynthetic rate per leaf area (A_{area}, monitoring the production of O_2) and (d) ratio of leaf carbon to nitrogen content (C:N). Mean values (dots) and standard deviations (error bars) are computed using data from the three corresponding tanks ($n = 12$). The significance levels are as follows: ns, $p > 0.1$; †, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$. See Table 1 for statistical details

Biomechanical traits were measured on the leaves of four other ramets using shearing tests performed on a universal testing machine (Instron™ 5942, Canton, MA, U.S.A.) and following the protocol described in Ibanez et al. (2013). Shearing tests enable the measurement of the energy required to cut the leaf lamina with a razor blade. Immediately after collection, the youngest mature leaf of each ramet was taped to 3-mm spaced supports and cut perpendicularly to the midrib using the stainless steel blade of a straight razor. The razor blade was set at 20° to the horizontal. A complete cut was performed at a rate of 10 mm/min. A subsequent second pass (blank pass) was not performed, since previous tests on similar leaves showed insignificant friction forces during the measurements (see Ang et al., 2008 for details). The specific work to shear (kJ/m^2) was computed as the integral of the force-displacement curves (area under the curves) divided by the area of the leaf cut section (Onoda et al., 2011). Images of the cut sections were taken immediately after the test using a binocular magnifier and a digital camera (Leica MC170 HD). They were then analysed with Leica Application Suite 4.3.3 software to measure the leaf cross-sectional area (m^2). For simplicity, we will refer to the specific work to shear as leaf toughness in the following sections.

Finally, four other ramets per tank were harvested to measure the leaf carbon to nitrogen ratio (C:N hereafter). All the mature and undamaged leaves were collected and oven dried at 65°C for 48 hr. Then, the leaf samples were manually ground into a fine powder. The total carbon and total nitrogen were measured on *c.* 2 mg samples using an elemental analyser (FlashEA; Thermo Fisher Scientific, Waltham, MA, U.S.A.).

2.3 | Data analyses

The statistical analyses were performed using R software (R Core Team, 2020). The effect of the ALAN treatment was tested for each trait (A_{area}, LMA, leaf toughness, and C:N) and each species (*M. verticillatum*, *P. coloratus*, and *V. spiralis*) separately. We used linear mixed models, accounting for trait variability due to tank identity, described as follows:

$$Y_{i,j} = \mu + \alpha_i + u_j + \varepsilon_{i,j} \quad (1)$$

where $Y_{i,j}$ is the trait value under treatment i in tank j, μ is the trait mean in the control, α_i is the treatment fixed effect ($i \in [1,2]$), u_j is the tank random effect ($j \in [1{:}6]$), and $\varepsilon_{i,j}$ is the random error term. A restricted maximum likelihood procedure was used for parameter estimation. The significance of the treatment fixed effect was estimated by analysis of deviance table (type II Wald χ^2 tests). Values of the specific work to shear were $\log_{10}$ transformed before the analyses to fulfil the normality assumption and avoid heteroscedasticity.

3 | RESULTS

The LMA ranged from 8.4 g/m^2 (*P. coloratus* in the control) to 58.5 g/m^2 (*M. verticillatum* in the control; Figure 1a). Leaves of *M. verticillatum* produced under ALAN had marginally significantly lower LMAs than those produced in the controls, while no effect was found on this trait in the two other species (Table 2). Leaf toughness ranged from 0.08 kJ/m^2 (*V. spiralis* in the ALAN treatment) to 5.7 kJ/m^2 (*M. verticillatum* in the control; Figure 1b). No treatment effect was found in any of the studied species for this trait (Table 2). A_{area} ranged from 0.16 µmol O_2 m^{-2} s^{-1} (*P. coloratus* in the control) to 2.68 µmol O_2 m^{-2} s^{-1} (*M. verticillatum* in the ALAN treatment; Figure 1c). Leaves of *P. coloratus* produced under ALAN showed a significantly higher maximum photosynthetic rate than those produced in the controls (Table 2). No effect was found on this trait in the two other species. Finally, the C:N ratio ranged from 12.4 (*P. coloratus* in the ALAN treatment) to 50.9 (*M. verticillatum* in the control; Figure 1d). Leaves of *M. verticillatum* produced under ALAN

TABLE 2 Fixed effects estimated by linear mixed models performed for each trait and each species and statistics of the analysis of deviance table (type II Wald χ^2 tests)

		Fixed effects	χ^2	*df*	P
Leaf mass per area (g/m^2)					
Myriophyllum verticillatum	μ	42.1			
	α_{ALAN}	−10.8	3.55	1	**0.059**†
Potamogeton coloratus	μ	12.5			
	α_{ALAN}	0.4	0.18	1	0.670
Vallisneria spiralis	μ	13.3			
	α_{ALAN}	3.4	0.40	1	0.529
Specific work to shear (log_{10} transformed)					
M. verticillatum	μ	0.06			
	α_{ALAN}	−0.08	0.63	1	0.428
P. coloratus	μ	−0.29			
	α_{ALAN}	0.05	0.16	1	0.686
V. spiralis	μ	−0.72			
	α_{ALAN}	−0.04	0.60	1	0.438
Maximum photosynthetic rate per leaf area (μmol O_2 m^{-2} s^{-1})					
M. verticillatum	μ	1.14			
	α_{ALAN}	0.33	1.02	1	0.312
P. coloratus	μ	0.33			
	α_{ALAN}	0.29	5.05	1	**0.024***
V. spiralis	μ	0.52			
	α_{ALAN}	−0.16	1.98	1	0.160
C:*N*					
M. verticillatum	μ	34.3			
	α_{ALAN}	−12.6	6.74	1	**0.009****
P. coloratus	μ	20.3			
	α_{ALAN}	−0.7	0.07	1	0.794
V. spiralis	μ	21.3			
	α_{ALAN}	5.9	0.45	1	0.503

Note: μ is the mean value of the controls, and α_{ALAN} is the effect of the ALAN treatment (see Eqn. 1 in the Material and Methods section). χ^2 is the statistical value, df is the degree of freedom and P is the p value. Significant effects are in bold, and significance levels are as follows: †$p < 0.1$; *$p < 0.05$; **$p < 0.01$.

had lower C:*N* values than those produced in the controls, while no effect was found in the two other species (Table 2). Overall, no effect of ALAN was detected in *V. spiralis* on any of the four measured leaf traits (Table 2, Figure 1).

4 | DISCUSSION

To our knowledge, our study is one of the first to test the effects of realistic levels of peri-urban ALAN on plant leaf traits, which all represent different leaf functions. The tested illumination (0.05 μmol m^{-2} s^{-1}) was significantly lower than the range of values usually applied (0.08–2 μmol m^{-2} s^{-1}) to test the responses of photosynthetic organisms to ALAN (Ayalon et al., 2019; Grubisic et al., 2017; Hölker et al., 2015; Levy et al., 2020; Poulin et al., 2014; Rosenberg et al., 2019) but still corresponded to the highest values recorded in peri-urban freshwater ecosystems. Even at this low-intensity night-time light, two of the three studied species showed weak but significant differences in at least one leaf trait after 5 months of exposure. Although weak, trait responses were consistent for both species: individuals exposed to ALAN produced leaves with higher photosynthetic capacities and lower C:N. In contrast, no or marginally significant effects were detected for LMA and leaf toughness. These results suggest that ALAN, even at a low intensity, induces plant physiological responses in these species, while other components of the leaves, such as their anatomical structure, may remain unchanged. Although not monitored here, one can expect that such an increase in photosynthetic capacity enhances plant growth, consistent with previous findings on four species of the *Poaceae* family (Flowers & Gibson, 2018) and *Asclepias syriaca* (Hey et al., 2020), which demonstrated a higher growth rate among plants exposed to ALAN. Indeed, these variations in leaf traits can result in higher carbon uptake during the day, enhancing plant growth. However, whether ALAN can be used as a resource for night photosynthesis remains to be tested under realistic conditions of illumination at night.

Overall, we demonstrated that dim light at night can be used by plants as an environmental cue (light as information sensu Gaston et al., 2013). Briggs (2006) suggested that ALAN could be interpreted as a shaded environment by triggering the corresponding photosystems. The decreased C:N ratio observed in our study for *M. verticillatum* supports this hypothesis, as C:N reduction is a common plastic response to low light conditions in submerged freshwater plants (Cronin & Lodge, 2003; Going et al., 2008; Li et al., 2005) and has been associated with an increase in leaf chlorophyll concentrations to maximise photosynthetic capacity (Barko & Smart, 1981; Barko & Filbin, 1983; Goldsborough & Kemp, 1988; but see Cronin & Lodge, 2003; Zhang et al., 2010 for contrasting results). Whether the same physiological pathways are involved under ALAN conditions (including the increase of chlorophyll concentration) remains to be tested and is a promising avenue for future research. The small effects observed on leaf anatomy and toughness are surprising, since several studies have showed lower leaf thickness and lower LMA under shaded conditions (Going et al., 2008; Goldsborough & Kemp, 1988). We hypothesise that physiological and anatomical responses result from complex or independent pathways. More precisely, they could involve contrasting sensitivity thresholds to ALAN.

Another important result in our study is the lack of response observed in *V. spiralis*. In the context of ALAN, differences in response intensity between species or even opposite responses have been reported for plant phenology (Cathey & Campbell, 1975b). More generally, plant plasticity to environmental conditions is known to differ strongly between species, but its determinants remain unclear. We

hypothesise that our observations result from differences in the species growth forms and ecological preferences. In particular, *P. coloratus* generally occurs in oligotrophic groundwater-supplied environments (Amoros et al., 2000), which tend to present clearer water due to the lower concentration of phytoplankton. Titus and Adams (1979) showed that *Myriophyllum spicatum* and *Vallisneria americana* (two species related to our study species) had different photosynthetic responses to a light gradient, with *V. americana* having the highest photosynthetic rate at low light intensity and *M. spicatum* being the most efficient species at high light intensity. In association with strong differences in canopy structure, they concluded that *V. americana* is better adapted to shaded environments, while *M. spicatum* favours lighted environments closer to the water surface. These differences in ecological preferences could lead to various sensitivities to light signals, resulting in contrasting responses to ALAN. Other aspects, such as phylogenetic constrains could be explored, but requires testing the plant responses on a higher number of species.

Interspecific variation in the plant response to ALAN may have profound effects on community structure and ecosystem functioning. Supporting this idea, Bennie et al. (2018) found that ALAN alters the species composition of grassland vegetation, and Hölker et al. (2015) found that ALAN favours photoautotrophic organisms in aquatic microbial communities. Here, we found that the species ranking based on their photosynthetic capacities was changed under the ALAN treatment due to contrasting responses: *V. spiralis* had a higher A_{area} than *P. coloratus* in the control treatment, while no significant difference between these species was found in the ALAN treatment (statistics not shown). More studies are needed to test whether trait responses corresponding to our observations can modify the competitive interactions between plant species, hence affecting the community composition. Moreover, high values of LMA, leaf toughness and leaf C:N are related to high plant defence and low quality for herbivores (Cronin et al., 2002; Elger & Willby, 2003). Contrasting trait responses to ALAN can thus affect plant–herbivore interactions, which are a major factor shaping the community structure (Lodge, 1991; Lodge & Lorman, 2011 for freshwater examples). Finally, both trait responses and changes in species composition would result in different community trait values, hence affecting ecosystem functioning. Hölker et al. (2015) found that a 5-month exposure to ALAN reduced microbial community respiration at night in freshwater sediments and suggested that it could affect net ecosystem productivity.

5 | CONCLUSIONS

Most of the current studies testing the effect of ALAN on photosynthetic organisms (from cyanobacteria to trees) apply nocturnal illumination that is rarely found apart from direct exposure to streetlights (PAR > 1 μmol m^{-2} s^{-1}). Therefore, the effects of ALAN must be tested in other ecological contexts since light pollution extends tens of kilometres from urban centres at much lower light intensities. We demonstrated that a 5-month exposure to an ALAN intensity corresponding to peri-urban areas (PAR < 0.1 μmol m^{-2} s^{-1}) has little effect on plant leaf traits but appears to trigger small physiological or chemical responses in the leaves of two species. Our results need to be compared with future studies to confirm the observed trends, especially under natural conditions (including variation in temperature, photoperiod, nutrient availability, and water turbidity) and with longer exposure. Although weak, these plant responses may have effects on community structure and ecosystem functioning.

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CONFLICT OF INEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study is openly available in the Zenodo Repository (https://doi.org/10.5281/zenodo.5518967).

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